Hypoglycemic Effects of 1-(p-chlorobenzenesulfonyl)-3-n-propylurea

Observations in Man

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This is a preliminary report of the clinical effects of 1-(p-chlorobenzenesulfonyl)-3-n-propylurea (chlorpropamide) in human diabetic subjects. Pharmacological studies have demonstrated chlorpropamide to be hypoglycemic at doses which are nontoxic in rats and dogs on long-term administration.[1] In this communication attention will be directed to comparative hypoglycemic effects of chlorpropamide and tolbutamide; the relationship of drug dosage and serum level to blood sugar changes; and clinical results in thirty-five diabetic subjects receiving the drug for six weeks to six months.

MATERIALS AND METHODS

The hypoglycemic effect of single doses of 1.0 gm. of chlorpropamide was compared with results obtained with 1.0 gm. of tolbutamide[2] in ten diabetic subjects controlled on diet alone, or in combination with insulin or tolbutamide. Tests were performed in a basal state and blood sugars determined by the Folin Wu method before administering the drug and at hourly intervals for four hours after meals. Effects on fasting blood sugars of substituting a daily dose of 1.0 gm. of chlorpropamide for 1.0 gm. of tolbutamide were investigated in six patients with mild diabetes being maintained on the latter drug.

The relationship of drug dosage and serum level to hypoglycemic effect was studied in six subjects by administering 1.0 gm. of chlorpropamide as a single dose orally and obtaining serial drug and blood sugar levels over a forty-eight-hour period. A comparison of peripheral and hepatic vein drug and sugar levels was made in five subjects using the technic of hepatic vein catheterization.[3] Results of prolonged administration of chlorpropamide on blood concentration of the drug were studied by weekly determinations in ten patients receiving maintenance dosage of 1.0 or 0.5 gm. of the drug each day. Chlorpropamide was determined in the serum by an ultraviolet spectrophotometric procedure.[4] The effects of the drug on glycogen storage[5] and glucocorticoid-induced hyperglycemia were studied as a part of an investigation of hepatic activity of this drug.[6]

The clinical usefulness and toxicity of chlorpropamide were investigated in eighteen patients with mild diabetes, seven with moderate diabetes, five with severe diabetes and three with brittle diabetes; patients were classified on the basis of their previous insulin requirements and response. Patients controlled on diet and tolbutamide, or less than 20 units of insulin, were considered mild; those requiring 20 to 60 units of insulin were considered moderate; those with maintained hyperglycemia and glycosuria on 60 or more units of insulin were considered severe. Diabetes was considered brittle when there was unpredictable fluctuation between hyperglycemia-glycosuria-ketonuria and insulin shock.[7,8] Two of the patients with mild diabetes who were previously controlled on tolbutamide were no longer responsive to this drug at the time chlorpropamide therapy was initiated.

During the earlier part of the investigation, each of the subjects received 1.0 gm. of chlorpropamide daily; subsequently, the daily dosage varied between 0.25 and 0.5 gm. depending upon its effectiveness. The level of diabetic control was determined by symptoms, weight changes, glycosuria, and blood sugar levels each week. White and red blood cell counts, urinalyses, cephalin flocculation tests, and bromsulfalein tests were obtained to evaluate hematological, renal, and hepatic effects.

OBSERVATIONS

Tolerance tests showed chlorpropamide to produce a slightly greater hypoglycemic effect than tolbutamide after a single dose of the drug in eight of ten patients

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(figure 1). The magnitude of the difference in hypoglycemia was much smaller than that reported in dogs[9] and was not considered clinically significant. Nevertheless, substitution of 1.0 gm. of chlorpropamide for 1.0 gm. of tolbutamide produced a definite lowering of the range of fasting blood sugars (see Case 1) in each of six patients, one of whom developed marked hypoglycemia after the transfer.

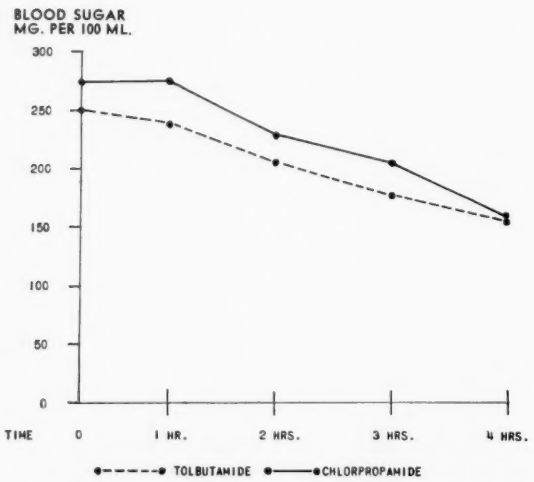

FIG. 1. Comparative effect of 1.0 gm. of tolbutamide and 1.0 gm. of chlorpropamide by mouth in a typical subject.

Serial studies of simultaneous blood sugar and drug levels showed an inverse relationship (figure 2). Absorption of chlorpropamide was rapid, and the peak serum level of the drug was usually reached within four hours. A therapeutic level was usually maintained for twenty-four hours and was often still present at forty-eight hours. At the end of twenty-four hours, two patterns were noted depending upon the excretion rate of chlorpropamide: (a) The drug level decreased to less than 40 gamma per 100 ml. serum, and the blood sugar returned to original levels; (b) the drug level was maintained above 40 gamma per 100 ml. and the blood sugar remained low. Weekly drug and blood sugar levels in five ambulatory patients showed an initial accumulative effect of the drug and progressive decrease in blood sugar level with daily administration of 1.0 gm. of the drug. A similar pattern was noted in some subjects receiving 0.5 gm. of the drug (figure 3). Hepatic vein catheterization demonstrated a similar relationship of drug level to the blood sugar level in hepatic venous blood and showed an earlier and more profound decrease in hepatic venous glucose similar to that obtained with

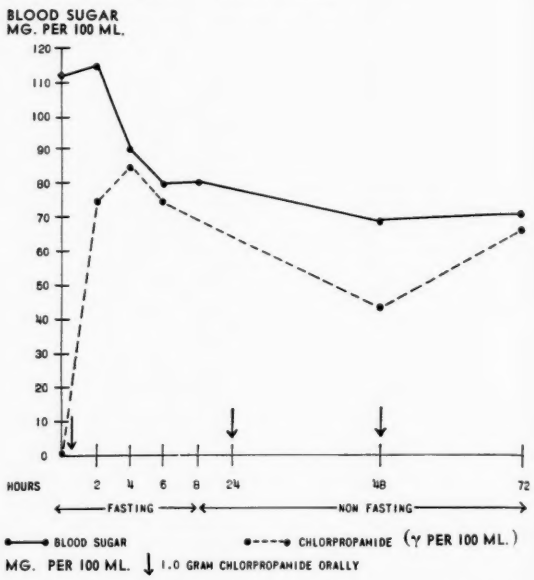

FIG. 2. Simultaneous blood sugar and chlorpropamide blood levels in a diabetic subject.

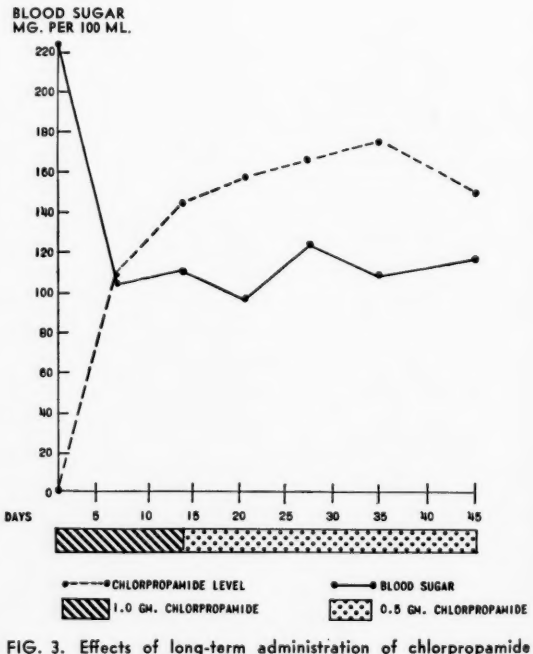

FIG. 3. Effects of long-term administration of chlorpropamide on drug and blood sugar levels.

insulin[10] and tolbutamide[3,11] (figure 4). A lag in drug level was noted in the hepatic venous blood suggesting initial storage of the drug occurs in the liver. Studies of the degree of epinephrine induced hyperglycemia before and after treatment with chlorpropamide showed a definite rise in some patients[5] (figure 5). Chlorpropamide often blocked the effect of glucocorticoids on glucose tolerance[12] (figure 6).

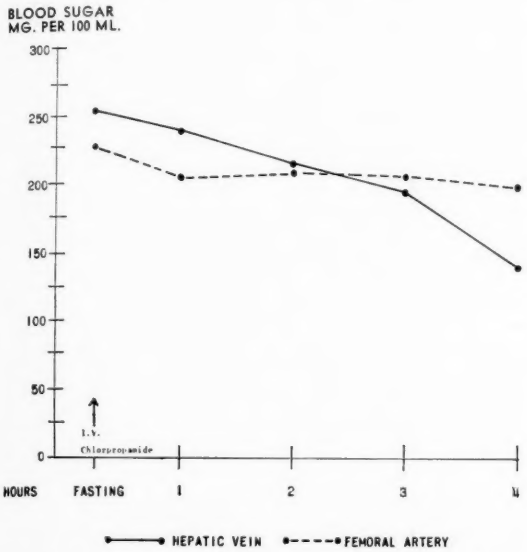

FIG. 4. Response of hepatic venous and femoral arterial glucose after 1.0 gm. of chlorpropamide intravenously.

Clinical studies showed that the drug controlled hyperglycemia and glycosuria, maintained an asymptomatic state and a satisfactory weight in fifteen of the eighteen mild diabetics and in six of the seven patients with moderate diabetes. It facilitated control in two patients with severe diabetes and one with brittle diabetes. Two patients who developed refractoriness to tolbutamide responded to chlorpropamide, and four patients with incomplete control on tolbutamide were better controlled with chlorpropamide. The usual maintenance dose was found to be 0.5 gm. daily.

Six patients, two of whom had nausea, had some degree of anorexia with doses of 1.0 to 2.0 gm. each day. Five patients developed temporary dizziness, attributed to blood sugar changes, which was controlled by decreasing the drug dosage. Three patients developed symptomatic hypoglycemia on the drug after receiving 1.0 gm. of it each day for two to four weeks. Blood sugars decreased to sixty, sixty-three and sixty-seven in patients who were forty, sixty and sixty-five years of age,

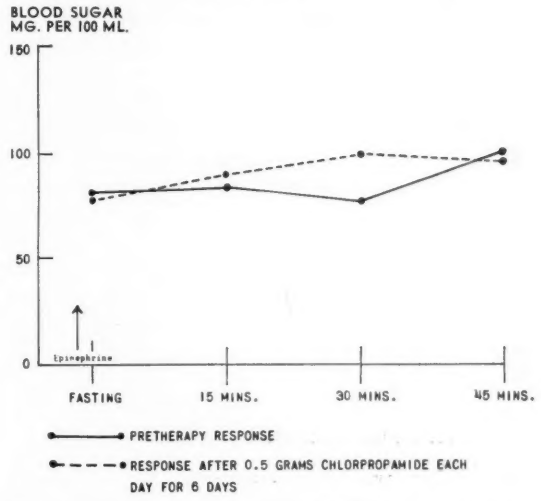

FIG. 5. Improvement in hyperglycemic response to epinephrine following chlorpropamide in a patient with a chronic viral hepatitis. There were no changes in the clinical state or other biochemical tests.

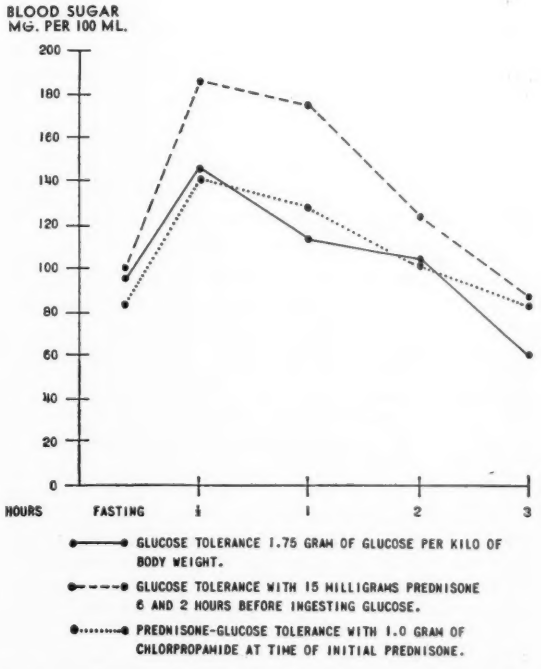

FIG. 6. Influence of chlorpropamide on glucose tolerance after administration of prednisone. Tests were performed at five-day intervals.

respectively. The hypoglycemia was characterized by sweating, palpitations and mental confusion. Hypoglycemia was treated with intravenous glucose in one patient. Oral glucose readily controlled symptoms in the other two patients. In the forty-year-old patient, reduction of the drug to 0.5 gm. every other day permitted satisfactory control; the older patients were best controlled receiving 0.5 gm. of the drug twice weekly.

One subject with mild diabetes and one with moderate diabetes whose blood sugars were well controlled on 1.0 gm. of chlorpropamide each day, lost weight and felt weak despite an adequate diet. These subjects gained weight and had a restoration of a sense of well-being on insulin. None of the patients exhibited any evidence of skin, hematological, renal or hepatic toxicity. The following case histories illustrate the various responses:

Case 1. Response with adequate control.

B. McD., No. 20093, a sixty-nine-year-old laborer, was hospitalized because of symptoms of prostatism and untreated diabetes. Laboratory studies showed a fasting blood sugar of 279 mg. per 100 ml., and a postprandial blood sugar of 510 mg. per 100 ml. Urinalysis revealed 2 per cent glycosuria and 1-plus acetonuria. His diabetes was controlled on diet and 20 units of NPH insulin, after which a retropubic prostatectomy was performed. Following discharge, he was maintained on a diet consisting of 180 gm. of carbohydrate, 80 gm. of protein, and 70 gm. of fat without insulin. After eight months of this therapy, at which time he had a fasting blood sugar of 263 mg. per 100 ml., and 4-plus glycosuria, the patient was given 2.0 gm. of tolbutamide followed by 1.0 gm. each day for maintenance. On tolbutamide his fasting blood sugar decreased to 185 mg. per 100 ml. Tolbutamide was replaced with 1.0 gm. of chlorpropamide daily. He became dizzy, had no glycosuria and a blood sugar was 140 mg. per 100 ml. The chlorpropamide was reduced to 0.5 gm. daily, and on this regimen he has been asymptomatic and has had satisfactory control of his diabetes during the past six months.

Case 2. Response with symptomatic hypoglycemia.

E. B., No. 078079, a sixty-five-year-old housewife, was admitted to the surgical service for resection of a carcinoma of the upper descending colon. Laboratory studies showed a fasting blood sugar of 320 mg. per 100 ml., 2 per cent glycosuria and acetonuria. Her diabetes was readily controlled with 180 gm. of carbohydrate, 80 gm. of protein and 70 gm. of fat and 30 units of crystalline insulin each day, and after resection of the colonic neoplasm she was maintained on this diet and 15 units of NPH insulin. After six months on this dosage of insulin, she had a blood sugar of 224 mg. per 100 ml., and 2 per cent glycosuria. Insulin was discontinued, and she was given 1.0 gm. of chlorpropamide daily. She became aglycosuric, and the dose of chlorpropamide was reduced to 500 mg. daily. After six weeks of therapy, the patient returned to the clinic complaining of dizziness, nervousness, sweating and headaches. She had voluntarily discontinued the drug two days previously. At the time of her clinic visit she had no glycosuria and a two-hour postprandial blood sugar was 69 mg. per 100 ml.

The drug was discontinued for one week and was then reinstituted at a dosage of 250 mg. daily. On this dosage she has remained asymptomatic. Weekly fasting blood sugars since that time have ranged around 120 mg. per 100 ml.

Case 3. No effect on severe diabetes.

C. G., No. 163959, a twelve-year-old schoolgirl with familial history of diabetes, developed progressive refractoriness to insulin after fair control on 30 units of NPH insulin daily. Her insulin dosage was gradually increased to 90 units each day. Despite insulin, a weighed diet and controlled exercise, her fasting blood sugar remained around 240 mg. per 100 ml., and postprandial blood sugars remained in the range of 415-480 mg. per 100 ml. To test the efficacy of an added sulfonylurea derivative, 1.0 gm. of chlorpropamide daily was given in addition to 90 units of NPH insulin. This was increased to 2.0 gm. daily with no evidence of alteration in her diabetic state. On this dosage she developed flatulence, constipation and abdominal pains. There were no other evidences of toxicity. These symptoms were not sufficiently severe to warrant discontinuation of the drug, and it was stopped because of a lack of response over an eleven-week period.

DISCUSSION

The ability of various sulfonamide derivatives to lower blood sugar depends upon their chemical configuration. Thus, propyl, isopropyl, butyl, isobutyl, tertiary butyl, amyl and ethyl-propyl compounds produce hypoglycemia, whereas, methyl, ethyl, texyl, and heptyl derivatives are inactive.[13] In searching for an ideal oral hypoglycemic agent for prolonged human use, it is necessary to determine the "therapeutic-toxic" ratio of each of the drugs demonstrated to lower blood sugar. Our initial experience suggests the 1-propyl derivative (chlorpropamide) is an effective hypoglycemic agent with the same range of toxicity in lower dosage as tolbutamide. In our experience it is more potent than tolbutamide when the same amount of these two drugs is given over a several-week period. Potential increased toxicity due to the presence of the chlorine molecule has not been demonstrated in this small group of patients.

This drug tends to accumulate due to its slow rate of renal excretion, lack of utilization, and perhaps increased tissue affinity. This provides a theoretical advantage as it insures greater constancy in drug level and facilitates stabilization of blood sugar. Temporary or continuous renal dysfunction may permit the drug to accumulate further so that unless very small doses are used, a profound hypoglycemia may result. For ideal control, it is desirable to obtain sulfonamide levels in addition to blood and urine sugar to establish the required maintenance dose. This dose may vary with alterations of diet, exercise, or the presence of infections or processes which influence hepatic and renal metabolism.

The mechanism of the hypoglycemia observed with sulfonylurea derivatives has been the subject of considerable controversy. It has been suggested that these materials may act primarily on the pancreas to stimulate release of insulin,[14] or decrease elaboration of glucagon,[15] or that they exert their effect within the liver by facilitating normal hepatic processes,[16] or inhibiting insulin antagonists.[17] Chlorpropamide appears to act similarly to both carbutamide and tolbutamide. Unlike phenethylbiguanide,[18] all of these drugs cause an increase in liver glycogen and this effect has proved useful in the management of hepatic disease associated with diabetes.[5,19]

The general indications for chlorpropamide are similar to those of other sulfonylurea agents. It is of greatest value in patients who can be controlled on a strict dietary regimen. The need for constant awareness that such drugs should be considered adjuncts to usual methods of control is shown by the two subjects who lost weight and developed weakness despite good control of their hyperglycemia and glycosuria on 1.0 gm. of chlorpropamide each day.

SUMMARY AND CONCLUSIONS

1. 1-(p-chlorobenzenesulfonyl)-3-n-propylurea (chlorpropamide) is an effective hypoglycemic agent in human diabetic subjects. This drug exhibited only a slightly greater hypoglycemic effect than tolbutamide after single 1.0 gm. doses of these drugs. However, it appeared to be significantly more potent when given on a daily maintenance basis. It permitted stabilization of diabetes in two patients who developed refractoriness to tolbutamide, and four patients with incomplete control on tolbutamide.

2. There is, in general, an inverse relationship between the level of chlorpropamide and its effects on peripheral and hepatic venous sugar. The drug tends to accumulate so that dosage adjustment is necessary to prevent a profound drop in blood sugar. Three patients on maintenance doses of 1.0 gm. daily developed severe hypoglycemia associated with mental confusion, tachycardia and sweating. Reduction of the amount and frequency of the administered drug prevented recurrent hypoglycemia.

3. Clinical studies in eighteen subjects with mild diabetes, seven with moderate diabetes, five with severe diabetes, and three with brittle diabetes, all of whom were treated with chlorpropamide over periods of six weeks to six months, showed this drug to be of value in management of most of the patients in the first two groups and of three patients in the last two groups. It did not produce any evidence of dermal, renal, hepatic or hemopoietic toxicity in these subjects during the period of study.

4. A regimen consisting of an initial dose of 0.5 gm. of chlorpropamide each day followed by a reduction or increase depending upon the clinical response provided quickest control and fewest side reactions. Slight gastrointestinal reactions were encountered in six subjects receiving 1.0 to 2.0 gm. of the drug each day. Best control was established by periodic blood sugar and sulfa levels.

SUMMARIO IN INTERLINGUA

Observationes In Re Le Effectos Hypoglycemic De 1-(p-chlorobenzenesulfonyl)-3-n-propylurea (Chlorpropamido) In Humanos

1. Chlorpropamido es un efficace agente hypoglycemic in humanos diabetic. Iste droga exhibiva un effecto hypoglycemic de grados solmente pauco superior a tolbutamido, quando le duo agentes esseva comparate post doses unic de un gramma. Sed chlorpropamido esseva significativemente plus potente que tolbutamido in administrationes diurne de mantenentia. Chlorpropamido permitteva le stabilisation de diabete in duo patientes qui habeva disveloppate refractorietate contra tolbutamido e in quatro patientes in qui tolbutamido effectuava un stabilisation incomplete.

2. Il existe, in general, un relation inverse inter le nivello de chlorpropamido e su effectos super le sucro venose peripheric e hepatic. Le droga tende a accumular se de maniera que un regulation del dosage es necessari pro prevenir un excessive reduction del sucro del sanguine. Tres patientes recipiente doses de mantenentia de un gramma per die disveloppava sever hypoglycemia associate con confusion mental, tachycardia, e sudoration. Le reduction del dosage e del frequentia del administration preveniva recurrentias del hypoglycemia.

3. Studios clinic in dece-octo subjectos con leve grados de diabete, septe con moderate grados de diabete, cinque con sever diabete, e tres con diabete fragile provava que iste droga esseva de valor in le tractamento del majoritate del patientes in le prime e le secundo gruppo e de tres patientes in le tertie e quarte. Le droga non produceva signos de toxicitate dermal, renal, hepatic, o hematopoietic.

4. Le plus rapide stabilisation e le minimo de reactiones lateral esseva effectuate per un regime que consisteva de un dosage initial de 0,5 g per die, sequite per un reduction o augmento secundo le responsa clinic obtenite. Leve reactiones gastrointestinal esseva incontrate in sex subjectos recipiente 1,0 e 2,0 g de chlorpropamido per die. Le melior methodo pro le effectuation del desirate stato de stabilisation esseva le determination

periodic del nivello sanguinee de sucro e de sulfa.

ACKNOWLEDGMENT

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Pentose Metabolism in Man

Recognition of a need for an understanding of the role of penetration phenomena in the metabolism of the muscle cell has resulted from the demonstration that the muscle cell membrane is responsive to insulin addition and deprivation, is a barrier to the free diffusion of sugars, and is, under certain conditions, the rate-limiting step in glucose utilization (R. Levine and M. S. Goldstein, *Rec. Prog. Hormone Res. 11*:343, 1954; C. R. Park, J. Bornstein and R. L. Post, *Am. J. Physiol. 182*: 12, 1955; E. J. Ross, *Nature,* London *171*:125, 1953). In order to study penetration phenomena, it is necessary to dissociate the processes of intracellular transport and intracellular utilization. This has been accomplished in a variety of laboratory animals by using several poorly metabolized pentoses as models for glucose in studies designed to elucidate the mechanisms of sugar transport and insulin action (M. S. Goldstein, W. L. Henry, B. Huddlestun and R. Levine, *Am. J. Physiol. 173*:207, 1953; E. Helmreich and C. F. Cori, *J. Biol. Chem. 224*: 663, 1957; D. M. Kipnis and C. F. Cori, *Ibid. 224*:681, 1957). Information concerning pentose metabolism, and the influence of insulin thereon in man, is fragmentary.

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Pharmacology of 1-(p-chlorobenzenesulfonyl)-3-n-propylurea (Chlorpropamide)

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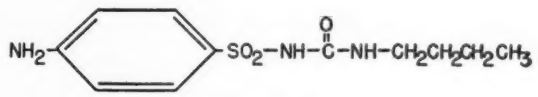
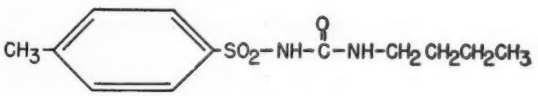
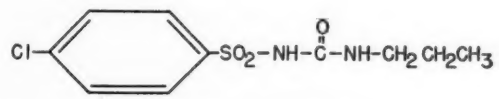
Chlorpropamide[1] is a hypoglycemic sulfonylurea compound closely related chemically to carbutamide and tolbutamide (figure 1). In animals chlorpropamide is more active milligram for milligram than either carbutamide or tolbutamide. This increase in potency is also found in diabetic patients.



CARBUTAMIDE



TOLBUTAMIDE



CHLORPROPAMIDE

FIGURE 1

METHODS

All animals used in acute studies were starved for eighteen hours. Chlorpropamide was administered orally in capsules, as tablets, or as a suspension in 5 per cent acacia. The suspensions were given by stomach tube. Solutions of chlorpropamide for parenteral application were prepared by adding enough 1 N sodium hydroxide or sodium carbonate to give a final *p*H of eight to nine.

Diabetes was produced in rabbits by the intravenous injection of alloxan monohydrate (Eastman) in a dose of 150 mg./kg. After recovery from the initial effects of the injection, the rabbits were maintained on daily injections of insulin. The rabbit used in this work had been diabetic for one year. Dogs were rendered diabetic by total pancreatectomy. After recovery from the operation they were maintained in metabolism cages, fed a weighed diet and given daily doses of insulin, powdered pancreas (pancreatin) and vitamins. Blood glucose was determined twice daily and twenty-four-hour urine sugar excretion was measured daily. Chronic administration of chlorpropamide was started thirty-seven days postoperatively.

Analytical methods. Blood glucose was determined by the Hagedorn-Jensen method,[2] and urine glucose by Benedict's quantitative method.[3] Serum concentrations of chlorpropamide were determined by a modification of the method developed by Spingler,[4] serum proteins by the biuret method,[5] serum alkaline phosphatase by the method of Kaplan and Warahara,[6] glutamic-oxalacetic transaminase by a modification of the method of Karmen,[7] and prothrombin time by the two-stage method of Ware and Seegers.[8]

RESULTS

Potency. In order to compare the hypoglycemic activity of different sulfonylurea compounds the substances were given to normal rats by mouth in a 5 per cent acacia suspension. Blood glucose was determined on tail vein blood samples one, two, three, five and seven hours after drug administration. The mg. per 100 ml. decrease from control was calculated and these values for four and six hours were estimated. The sum of these

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From The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana.

seven values was recorded as the *score* for each animal. This score gives a crude measure of the degree and duration of hypoglycemia produced by each drug and can be used to plot a dose-response curve. An example of the use of this method for determining potency is illustrated in figure 2.

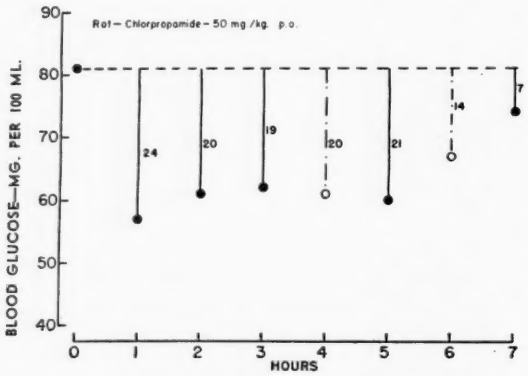

FIG. 2. Determination of the hypoglycemic score. The effect of a single oral dose of chlorpropamide in one normal rat. Solid circles are blood glucose values actually determined. Open circles are estimated values.

The mean score for eighteen rats treated with each of several doses of each drug was then used to calculate the dose-response curves for carbutamide, tolbutamide and chlorpropamide (figure 3). The slopes of the lines for carbutamide and tolbutamide are the same and the potency of carbutamide is slightly greater than that of tolbutamide. The slope of the chlorpropamide dose-response curve is steeper than that of the other two compounds. The meaning of this change in slope is not clear but, in the absence of any evidence for a different mechanism of action, it probably indicates a different rate of absorption, excretion or metabolism of this drug. Doses of chlorpropamide above 35 mg./kg. orally have greater hypoglycemic potency than either carbutamide or tolbutamide and the higher the dose the greater the difference until the plateau is reached at around 200 to 250 mg./kg.

A similar potency comparison was made in normal dogs. Here the dose range was greatly restricted because dogs are more sensitive to severe hypoglycemia. These dose-response curves for carbutamide, tolbutamide and chlorpropamide are shown in figure 4. In this species the slopes of the curves for carbutamide and chlorpropamide are the same but tolbutamide has a very flat

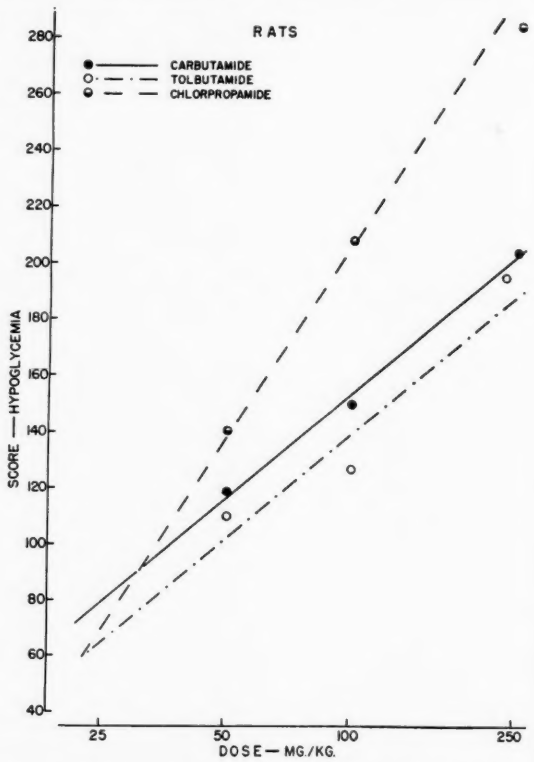

FIG. 3. Dose-response relationships of three sulfonylurea derivatives in normal rats. All compounds were administered orally by stomach tube.

Compound	Relative potency Per cent	95% confidence limits Per cent
Chlorpropamide	100	—
Carbutamide	54.0±6.3	42.9—68.0
Tolbutamide	45.2±6.7	33.8—60.6

slope; in fact, it was difficult to determine any dose-response relationship for tolbutamide in this species. Again, chlorpropamide was the most potent of the three compounds.

Serum concentrations of chlorpropamide. The method developed by Spingler[4] for the determination of blood concentrations of sulfonylurea compounds involves removal of the alkyl amine from the sulfonylurea, allowing this segment to react with dinitrofluorobenzene and measuring the color formed. This means that any measurements of serum concentration are presumptions only and may not be a true measure of unchanged compound, be it chlorpropamide, tolbutamide, or any other substituted sulfonylurea. However, this appears to be the best method available for easy determination of serum levels of these drugs and it gives results with chlorpropamide

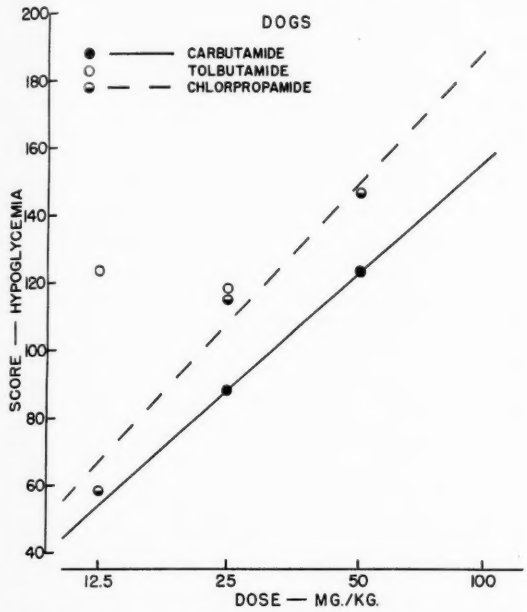

FIG. 4. Dose-response relationship of three sulfonylurea derivatives in normal dogs. All compounds were administered orally by stomach tube.

Compound	Relative potency Per cent	95% confidence limits Per cent
Chlorpropamide	100	—
Carbutamide	67.3±11.0	48.8—92.7
Tolbutamide	No dose-response relationship found	

that indicate good absorption of the compound although in dogs the blood concentration does not correlate very well with the degree of hypoglycemia produced.

When a single dose of chlorpropamide was administered orally to normal dogs, the serum concentration increased gradually and reached a peak within three to seven hours. The drug then disappeared from the blood at a very slow rate, measurable amounts still being present at ninety-six hours. In figure 5 are shown representative curves of serum concentration of chlorpropamide and blood glucose values for a normal dog given 500 mg. of drug (58.8 mg./kg.) as a single oral dose after an eighteen-hour fast.

When seven different normal dogs, weighing 7.4 to 10.5 kg., were given 500 mg. each of chlorpropamide by mouth, the mean fall in blood glucose at three hours was 8.8 per cent and the mean serum concentration of the drug was 23.4 mg./100 ml. The variation in drug level was not so great as was the variation in change of glucose concentration. The former ranged from 17.7 to 30.9 mg./100 ml. and the latter from +2 to —21 mg. per 100 ml. Four of these dogs had blood glucose concentrations at three hours that did not vary significantly from controls, whereas the falls for the three remaining dogs ranged from 15 to 21 mg. per 100 ml. Thus, with this one dose of drug there was very little correlation between the serum concentration of chlorpropamide and the effect on blood glucose.

In rabbits, chlorpropamide is absorbed rapidly, and the rate of disappearance from the serum is much faster than in the dog (figure 5). There was a trace of drug in the serum at twenty-four hours, but by forty-eight hours no chlorpropamide could be detected. The rabbit used for the experiment illustrated in figure 5 had alloxan diabetes and had received the last dose of insulin (2 units) about eighteen hours before the administration of 200 mg. of chlorpropamide. This combination of residual exogenous insulin and chlorpropamide caused a significant decrease of blood glucose even though the rabbit had severe diabetes and could be presumed to have few, if any, functional beta cells.

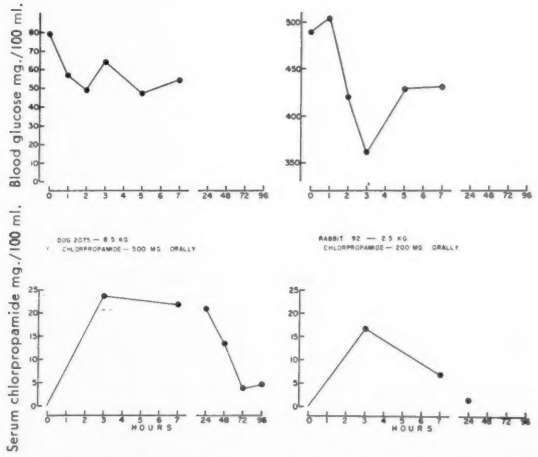

FIG. 5. Serum concentration of chlorpropamide and blood glucose response in a normal dog and an alloxan diabetic rabbit.

However, in dogs and rabbits the presence of functioning beta cells is not always necessary for the hypoglycemic action of carbutamide and tolbutamide. In some, but not all, depancreatized dogs several investigators have observed an enhancement of the action of exogenous insulin when either of these sulfonylurea compounds is administered.[9-48] The rabbit used in the experiment in figure 5 was one that responded to such a combination of treatment. Of three totally depancreatized dogs given 1.0 gm. of chlorpropamide eighteen hours after the last dose of NPH insulin, two responded with a

fall in blood glucose and the third exhibited only a rise. The results of these experiments thus follow closely those obtained previously with carbutamide and tolbutamide.

Acute toxicity. Acute toxicity of chlorpropamide was studied in mice and rats. The acute oral toxicity of the sulfonylurea compounds is difficult to assess because any dose that causes severe hypoglycemia may kill normal animals by the severity of its action. When these compounds are given orally to starved mice, the animals become depressed during the first few hours and often do not eat enough to counteract the hypoglycemia produced. This situation leads to marked irregularities in the dose-response data and also makes comparison between compounds difficult. In table 1 are data for toxicity studies in mice and rats with carbutamide, tolbutamide and chlorpropamide. Approximate $L.D._{50}$'s indicate that chlorpropamide is more toxic than the other two compounds, but this may be only a reflection of the greater hypoglycemic potency of chlorpropamide.

TABLE 1

Acute toxicity

		$L.D._{50}$ in mg./kg.		
Animal	Route	Carbutamide	Tolbutamide	Chlorpropamide
Mouse	i.v.	1,900	600	613
	oral	3,500	1,800	721
Rat	i.v.	980	—	—
	oral	1,030	3,000	920

Chronic toxicity. Groups of ten rats each received chlorpropamide in the diet for a period of five weeks. All the animals survived the entire period and complete autopsies at the end of the experiment did not reveal any gross or microscopic abnormalities. Blood studies at the end of the period showed normal values for hemoglobin, red cell count, white cell count and white cell differential. In table 2 are recorded the mean weight gains of rats on two concentrations of chlorpropamide in the diet. When the animals consumed an average of 38.5 mg. per day of drug (332 mg./kg./day) there was no inhibition of growth. When the drug concentration was doubled, the mean daily intake increased to 71 mg./day (752 mg./kg./day) and the growth rate was retarded. A similar decreased growth rate was produced by carbutamide when the concentration of drug in the diet was twice as great (1 per cent).[14]

TABLE 2

Chronic toxicity of chlorpropamide in rats (five weeks)

Concentration of drug in diet	Average body weight gain—gm.	Average daily drug intake—mg.
Control—no drug per cent	60.3	—
0.25	59.4	38.5
0.50	29.4	71.0

Three normal adult dogs, two normal puppies and two totally depancreatized dogs have been treated with daily doses of chlorpropamide for varying lengths of time. The duration of treatment for each animal (up to May 1, 1958) is given in table 3. Only two of the seven dogs have died, and one of these deaths (dog 9406) was due to severe chronic hypoglycemia. A complete autopsy of this dog did not disclose any pathological changes that could have been caused by the drug, or could have been responsible for the death.

Dog 9418 became severely anemic due to blood loss from intestinal bleeding and was killed for autopsy. Table 4 summarizes the results of the various clinical laboratory examinations made on this dog during the period of drug administration. The serum alkaline phosphatase was higher than usual during the control period in this dog. After ten to fifteen days of drug treatment it increased above the control level and remained elevated. The serum albumin concentration fell slowly throughout the period of treatment, but the total proteins of the serum did not decrease until the last week of life. The prothrombin time increased slightly but the elevation was not great enough to account for the intestinal bleeding of the terminal stages. However, during the last two weeks of life there was a marked increase in bleeding time and often it was difficult to stop bleeding from the ear veins after puncturing them to get blood for glucose determinations. The intestinal bleeding that appeared during the last five days was apparently from capillaries, and no lesions were found on postmortem examination of the intestines. The liver was light brown upon gross examination and microscopic examination showed small fat globules in many liver and Kupffer cells. This toxic effect is the same as that noted in several laboratories when diabetic dogs have been treated with large doses of either carbutamide or tolbutamide.[15-17]

In table 3, the mean serum concentration of chlorpropamide is given for each animal and the high and low values are also recorded. Blood samples for measurement of serum concentration were always taken just before the morning dose of drug was administered, therefore these values are the lowest reached each day. The concentration varied considerably from week to week, but the mean level in the normal dogs was twice that of the totally depancreatized animals. The one dog (9418) that

TABLE 3

Prolonged treatment of dogs with chlorpropamide

Dog	Dose mg./day	Days treatment	Body weight—kg. Start	Body weight—kg. May 1, '58	Serum alkaline phosphatase—units Start	Serum alkaline phosphatase—units May 15, '58	Drug concentration in serum—mg./100 ml. Mean	Low	High
9414 Normal	500	456	11.10	12.20	4.1	0.6	22.4	12.0	36.8
9406 Normal	500	201	10.70	11.70*	3.0	1.5	21.2	7.9	42.2
9403 Normal	500	456	9.70	9.70	3.0	0.6	27.1	8.3	41.2
9418 Depan.†	500	51	9.35	7.52‡	9.0	25.5	8.8	4.8	15.8
9416 Depan.	500	504	7.90	9.21	6.5	7.3	10.1	5.3	14.5
2448§ Normal	125‖	58	2.68	6.70	5.8	3.5	8.4	5.2	11.3
2449§ Normal	125‖	58	4.34	8.98	4.1	2.9	11.5	9.2	13.3

*Aug. 15, 1957. This dog died on Aug. 20, 1957, during severe hypoglycemic convulsions.
†Depan.—totally depancreatized.
‡Feb. 3, 1957. This dog became moribund from anemia and emaciation and was killed for autopsy. For details see text.
§Puppies (littermates) were thirteen weeks old when drug treatment was started.
‖Dose was increased to 187.5 mg. per day on April 23, 1958.

TABLE 4

Dog 9418, totally depancreatized, clinical chemistry during treatment with chlorpropamide

Period	Treatment	Body wt. kg.	Fasting blood glucose —mg. per 100 ml.	24-hour urine sugar —gm.	Serum conc. of drug mg./100 ml.	Serum proteins per cent Total	Serum proteins per cent Albumin	Serum alkaline phosphatase units	Prothrombin time—seconds	Insulin—NPH units/day
Nov. 15-24	Control	9.47	368	24.84	—	5.30	3.45	9.2	22.0	9.2
Nov. 25-Dec. 4	Control	9.15	303	23.03	—	5.15	3.04	10.3	27.0	8.6
Dec. 5-14	Control	9.26	248	11.75	—	6.90	2.40	9.0	20.0	9.0
Dec. 15-24	Chlorpropamide 500 mg./day	8.79*	192	4.46	15.8	5.00	2.40	8.3	30.0	5.9
Dec. 25-Jan. 3	"	8.08	267	8.85	8.9	5.35	2.33	15.5	33.4	6.5
Jan. 4-13	"	7.75	295	12.36	7.9	5.35	1.95	20.0	32.8	8.4
Jan. 14-23	"	7.58	244	12.85	6.4	5.30	1.60	30.9	37.0	9.2
Jan. 24-Feb. 3	"	7.42	211	6.69	7.2	3.30	1.50	25.5	—	9.2

*Sudden weight loss caused by birth of three puppies.
Increased bleeding time first noted on January 14 and blood first observed in stool on January 31. Killed for autopsy on February 3.

became moribund as a result of a toxic action of the drug had the lowest mean drug concentration of the group. The reason for the lower serum concentrations in the diabetic animals has not been established.

DISCUSSION

Chlorpropamide is a sulfonylurea derivative closely related to carbutamide and tolbutamide. It causes hypoglycemia when administered orally or parenterally to normal animals. It does not affect the blood glucose concentration in totally depancreatized dogs or severely alloxan diabetic rabbits when no insulin is administered. However, in both types of diabetic animals chlorpropamide will frequently increase the hypoglycemic effectiveness of small amounts of exogenous insulin.

Because of the chemical similarity between chlorpropamide and the other, extensively studied, sulfonylurea compounds, we have assumed that the mechanism

of action is the same and we have not repeated the many experiments performed in an attempt to delineate the role of carbutamide and tolbutamide in carbohydrate metabolism. However, in none of our experiments has there been any evidence for a different mechanism of action.

The activity of chlorpropamide is qualitatively the same as that of tolbutamide or carbutamide, but quantitatively the new sulfonylurea derivative is much more active than either of the previously studied substances. The hypoglycemic potency of chlorpropamide appears to be about twice that of carbutamide, at least in dogs. In rats the slope of the chlorpropamide curve is steeper than that of the other two compounds, so that a potency comparison is less useful. However, at a dose of 50 to 100 mg./kg. in the rat, the potency is about twice that of carbutamide.

The acute toxicity of chlorpropamide is also greater than that of carbutamide or tolbutamide. If acute toxicity includes any deaths occurring during twenty-four or forty-eight hours after drug administration, then the toxicity figure for any sulfonylurea includes the deaths caused by extreme or prolonged hypoglycemia. If the compound being studied has a marked and prolonged hypoglycemic action at low doses, the acute $L.D._{50}$ will inevitably be low. It is likely that this is part of the reason for the increase in toxicity of chlorpropamide as compared with carbutamide and tolbutamide.

Chlorpropamide is readily absorbed from the gastrointestinal tract. Serum concentrations of the drug do not correlate well with hypoglycemic action. The rate of disappearance of the drug from the serum is extremely slow in dogs. After ninety-six hours a measurable amount of chlorpropamide is still present. In rabbits, however, the serum concentration falls much more rapidly and only a trace remains at twenty-four hours. These species differences in rate of metabolism or excretion of chlorpropamide are similar to those observed with carbutamide and tolbutamide. The duration of the hypoglycemic activity and of effective serum concentrations of drug is more prolonged than when tolbutamide is administered. This combination of greater hypoglycemic potency and greater duration of effective blood concentrations should make the compound active in the control of mild diabetes at a daily dose considerably lower than that required with tolbutamide.

SUMMARY

Chlorpropamide is a sulfonylurea derivative that produces hypoglycemia when administered orally or parenterally to normal rats, mice or dogs. It is about twice as active as carbutamide. The $L.D._{50}$ in mice upon oral administration is 721 mg./kg. and in rats is 920 mg./kg. Daily administration to normal rats for five weeks of 0.25 and 0.50 per cent in the diet has not produced any pathological lesions, although the animals on the higher dose have shown a decreased rate of growth. Chronic administration to normal dogs has not caused any abnormalities. One of two totally depancreatized dogs receiving chlorpropamide daily died after fifty-one days with increased bleeding time and other indications of liver dysfunction.

Chlorpropamide is readily absorbed and it takes several days for the serum concentration to fall to zero after a single large dose in a dog. In rabbits the drug is metabolized or excreted more rapidly.

SUMMARIO IN INTERLINGUA

Le Pharmacologia de 1-(p-chlorobenzenesulfonyl)-3-n-propylurea (Chlorpropamido)

Chlorpropamido es un derivato de sulfonylurea que produce hypoglycemia quando administrate per via oral o parenteral a normal rattos, muses, o canes. Illo ha circa duo vices le activitate de carbutamido. Le DL_{50} in muses post administration oral es 721 mg/kg e in rattos 920 mg./kg. Le administration diurne a rattos normal durante cinque septimanas in un concentration de 0,25 e 0,50 pro cento in le dieta non ha producite ulle lesiones pathologic, ben que le animales recipiente le plus alte del duo concentrationes manifestava un relentate crescentia. Le administration chronic a canes normal non resultava in ulle anormalitate. Un de duo totalmente dispancreatisate canes que recipeva chlorpropamido in doses diurne moriva post cinquanta-un dies con augmento del tempore de sanguination e altere signos de dysfunction hepatic. Chlorpropamido es facilemente absorbite.

Post un sol dose massive le concentration del droga in le sero de canes retorna a zero solmente in le curso de plure dies. In conilios le droga es metabolisate o excernite plus rapidemente.

ACKNOWLEDGMENT

We wish to acknowledge with gratitude the assistance rendered us in the conduct of this work: Dr. Paul N. Harris for performing the autopsies and making the histological studies; Mrs. I. K. Erdman for the serum transaminase determinations; Mr. C. L. Rose and Mr. R. D. Fink for determinations of prothrombin time. We are especially indebted to Dr. F. G. Henderson for performing the total pancreatectomies on our dogs.

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Histidine and Experimental Atherosclerosis

A report from Russia mentions briefly the various substances that have been used in that country for the treatment of atherosclerosis, and describes an experiment with rabbits which suggests that histidine may be one more substance that will lower the blood cholesterol level.

Chimakadze (*Farmakologiia i Toksikologiia* 17:11, 1954) was led to study the influence of histidine on atherosclerosis as a result of the recognized property of histidine in suppressing adrenergic innervation, and his preliminary observation that rabbits with high levels of cholesterol show a reduced level of sympathin (an assumed hormone excreted by smooth muscle which causes an increased heart rate) in blood.

In his experimental work, Chimakadze (loc. cit.) fed twenty rabbits cholesterol (0.2 gm. per kg.) dissolved in peach kernel oil. After being maintained on this regimen for forty-five days, one half of the animals had their intake supplemented with 80 mg. of histidine per day (optical isomer not stated). The experiment was continued for an additional twenty days.

In the rabbits on the basal ration, the blood cholesterol level increased progressively until it reached a value of 3,846 mg. per cent by the sixty-fifth day. The blood pressure, which was determined in a carotid skin loop, increased only slightly. Morphological examination of the blood vessels indicated an extensive atherosclerosis in those animals receiving only the cholesterol.

The rabbits receiving the histidine in addition to the cholesterol showed a marked reduction in the blood cholesterol level. This was apparent by the tenth day of the histidine therapy, when the level had been reduced by 600 to 800 mg. per cent. On the twentieth day, the level had dropped by 1,000 to 1,300 mg. per cent. The reactivity of the circulatory system to adrenalin was also restored to normal following the histidine administration. Morphological examination of the blood vessels indicated a very mild degree of lipid infiltration of the intima. Atherosclerotic plaques were seen in the abdominal aorta in only one of the rabbits receiving histidine.

The above observations are interesting in suggesting that a relatively small amount of histidine has a very pronounced effect on the blood cholesterol level of rabbits. If a similar phenomenon occurred in man, it would mean that 2.8 gm. of histidine per day should be effective in a 70 kg. man.

From *Nutrition Reviews,* Volume 16, Number 7, 1958, pp. 213-14.

Endocrine Studies in Pregnant Diabetic Women

E. Jurgen Plotz, M.D., M. Edward Davis, M.D., and Henry T. Ricketts, M.D., Chicago

Thirty-five years' experience with the use of insulin has resulted in a notable increase in life expectancy and fertility of young diabetic women, a reduction in maternal mortality almost to zero and a substantial diminution of fetal loss. Pregnancy in diabetes, however, is still associated with an excessive fetal mortality for reasons many of which remain obscure. Factors that are known to militate against fetal survival are diabetes of long duration and unstable character, toxemia and oversized babies. But the mechanisms by which these operate to destroy the infant are unknown.

The possible relationship of these clinical observations to the profound changes in the hormonal environment during pregnancy has attracted the interest of numerous investigators. White and Hunt[1] first reported an improved fetal survival rate in a large number of diabetic pregnancies in which treatment with progesterone and estrogens was employed. Their concept of management was based on theories of Smith and Smith[2] who claimed that an imbalance of the pregnancy hormones, progesterone, estrogens, and chorionic gonadotropin, is prevalent in diabetic women during gestation. More recently, Hoet[3] has raised the question of whether increased activity of adrenal cortical hormones, with their diabetogenic and salt- and water-retaining properties, may be partly responsible for some of the complications observed. There is indeed, even in normal pregnancy, good evidence for an increased production of such hormones, the source of which is not yet clearly established. Larger than normal amounts of cortisol, cortisone and their metabolites appear in the urine, and the concentration of corticosteroids in the blood is elevated. It is reasonable to suspect that such changes might have significant effects on the already disturbed metabolism of the pregnant diabetic.

Published accounts of attempts to relate changing patterns of adrenal steroid excretion as gestation progresses to alterations in the state of pregnancy and of diabetes are rare. This study is concerned with such an effort.

The urinary excretion of certain steroids in diabetic pregnant women has been compared with that in healthy pregnant women and correlated with the following: (1) insulin requirement, (2) control of diabetes, (3) presence of vascular complications, (4) maternal toxemia, weight changes and water retention, and (5) fetal survival. Unfortunately, these investigations were carried out before the significance of aldosterone was generally appreciated and before methods for its determination were available.

In addition, balances of sodium, chloride, potassium, phosphorus and nitrogen were determined. Since all of these showed the positivity to be expected in the pregnant state, and since the minor variations observed could not be correlated with changes in urinary steroid excretion, they will not be discussed further.

MATERIAL AND METHODS

Thirteen pregnancies in ten diabetic women were investigated. One patient had three, and another two, consecutive pregnancies while under study. Age of the patient, duration and control of diabetes, presence of vascular complications, and data concerning the fetal weight and survival are listed in table 1.

The patients were hospitalized several times during each pregnancy for study for periods varying from ten to fourteen days. They were under the close supervision of the prenatal and metabolism clinics in the intervals between hospitalizations. In the hospital, all patients were given weighed constant diets with two exceptions: The caloric intake was reduced from 2,010 to 1,680 calories during midpregnancy in one patient (S.R.), and a salt-free diet was prescribed for another patient (I.M.) who developed toxemia of late pregnancy. Measured amounts of sodium chloride ranging from 1.4 to 6 gm. per day were added to the weighed diets so as to bring the total salt intake to 6.9 to 12.4 gm. daily. During the last weeks of pregnancy, the amount of added salt was reduced or omitted in six cases because of excessive weight gain. The patients drank measured amounts of distilled water. Body weights were recorded daily by means of a sensitive beam balance.

Twenty-four-hour urine specimens were collected without preservatives, were kept during collection in a small icebox in the patient's room and were then taken immediately to the laboratory. All collections were checked

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TABLE 1

Patient	Age (years)	Duration of diabetes (years)	Vascular disease	Control during pregnancy	Duration of gestation* (weeks)	Fetal weight (gm.)	Fetal survival
E.S.1	33	3	—	Good	36	3,660	Survived
E.S.2	35	5	—	Fair—good	37†	4,270	Died in utero
S.C.	24	3	—	Excellent	36	4,165	Survived
B.W.	22	3	—	Fair—good	37	3,995	Survived
S.H.	25	7	—	Good	36	4,080	Survived
M.C.	20	6	—	Excellent	36	4,355	Survived
P.C.	22	9	—	Fair	35	4,185	Survived
S.R.	24	10	—	Good	36	2,510	Survived
J.D.	24	14	(+)‡	Poor	36§	1,715(mac.)	Died in utero
I.M.	30	21	—	Poor	32	3,220	Died post partum
J.B.1	30	18	+	Fair	33	3,280	Died post partum
J.B.2	31	19	+	Good—fair	32½	3,365	Died post partum
J.B.3	32	20	+	Poor	37	3,850	Survived

*Calculated from day of conception.
†Died in utero shortly before delivery.
‡Only one microaneurysm seen.
§Died in utero during the thirty-first week.

for completeness by creatinine determinations. Urinary corticoids were estimated by a modified method of Corcoran and Page[4] and crude neutral 17-ketosteroids by the Holtorff and Koch modifications of Zimmermann's method,[5] using dehydroisoandrosterone acetate as standard with values expressed in terms of dehydroisoandrosterone. Methods for the determination of pregnanolone, androsterone, etiocholanolone and 11-oxygenated 17-ketosteroids have been previously detailed.[6]

RESULTS AND DISCUSSION

Corticoids. During the first trimester of normal pregnancy, urinary corticoids gradually increase above values found in nonpregnant women. They then fall to normal, but between the nineteenth and twenty-first week rise again to rather high levels with a peak late in the third trimester.

In the thirteen diabetic pregnancies here reported, wide fluctuations of the daily corticoid excretion were observed during the first and third trimester; their average values, however, did not differ significantly from those found in normal gestation. In contrast, both the average (figure 1) and, in eleven of thirteen pregnancies, the individual (table 2) urinary corticoids determined during the thirteenth to twenty-fourth week were significantly higher than in normal pregnant women.

Insulin Requirement. Assuming that the urinary excretion of corticoids is a true reflection of the activity of adrenal cortical hormones, one would expect an increasing insensitivity to insulin as pregnancy advances. A tendency in this direction was, in fact, observed in all our patients. This finding is in accord with reports by White,[7] Pederson,[8] Hoet,[8] and others.

A larger requirement for insulin, however, is not predictable in the individual patient. In ten of the thirteen pregnancies the insulin dose had to be raised during the second trimester (table 2), a time when in normal pregnancy the urinary corticoid excretion returns to values observed in nonpregnant individuals. Among the eleven pregnancies characterized by high corticoid excretion during the second trimester, a larger insulin dosage was necessary in eight. However, the insulin requirement during that time also rose in the two pregnancies characterized by low (normal nonpregnancy) values for

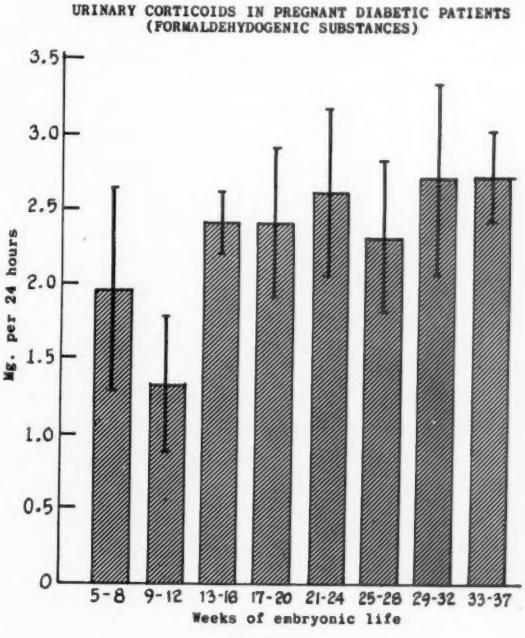


FIGURE 1

TABLE 2

Correlation of urinary corticoid excretion with insulin requirement in pregnancy

Corticoids		Insulin	
Second Trimester			
Within limits of normal pregnancy	2	Definite increase	2
Abnormally high	11	Definite increase	8
		Decrease (to non-pregnancy values)	3
Third Trimester			
Within limits of normal pregnancy	6	Increase	6
Abnormally high	6	Increase	6
Abnormally low	1	Increase	1
Period Shortly Before Delivery*			
Further increase	4	Further increase	1
		Decrease†	2
		Decrease (to non-pregnancy values)	1
Decrease‡	5	Decrease†	4
		Increase	1
No change	1	Decrease†	1

*Three patients were not hospitalized during this period.
†Insulin requirement after decrease still higher than before pregnancy.
‡Corticoid value after decrease still above normal non-pregnancy values.

corticoid excretion. A positive correlation between corticoid excretion and insulin dosage was observed in the majority of the cases during the first part of the third trimester, but it was negative in five of ten pregnancies during the period shortly before delivery (table 2).

There are several possible explanations for the failure of uniformly positive correlations:

1. It may be that the method employed for the determination of corticoids was not adequate. There is no doubt that all chemical methods for the estimation of a mixture of various steroids in urine have their shortcomings.

2. The urinary excretion of steroid hormones and their metabolites does not always reflect the true state of activity of the endocrine gland in question.

3. Other hormones produced in increased or decreased amounts during pregnancy may modify the influence of adrenal cortical hormones on carbohydrate metabolism. For instance, growth hormone, produced in all likelihood by the maternal pituitary, is at least as diabetogenic as the hormones of the adrenal cortex. Using a bio-assay method, Gemzell et al.[9] found the highest concentration of growth hormone in the retroplacental blood of a diabetic woman. The exact role of other steroids that might affect carbohydrate metabolism and are produced during pregnancy in large amounts (estrogens, progesterone) is not well understood. In animal experiments natural and synthetic estrogens can produce a "steroid diabetes" with all the characteristics of that elicited by adrenal cortical hormones.[10]

4. Although the claim of early investigators that insulin produced by the fetal pancreas may ameliorate the diabetes of the mother has not been conclusively established, the possibility still remains that fetal insulin may contribute to the maternal supply in the later stages of pregnancy and thus affect the demand for exogenous insulin.

Control of diabetes during pregnancy. The control of diabetes was estimated on the basis of blood and urinary glucose values obtained during both hospital and home periods and was classified as excellent, good, good to fair, fair to good, fair or poor. There was, during most periods, a positive correlation between poor control and the amounts of corticoids excreted in the urine (table 4). The data permit no definite conclusion as to which was cause and which effect, but with the diabetes in a reasonably stable state, it would seem likely that the augmented production of adrenal steroids common to pregnancy was the primary factor. Extremes in the state of the diabetes, however, are another matter. For example, when a ketoacidosis of moderate degree developed in one patient (J.D.), the urinary excretion of corticoids was extremely high. This observation is in accord with the findings of Wolfe and Paschkis[11] and McArthur et al.,[12] who reported an increased adrenal cortical activity in nonpregnant diabetics during acidosis. Perkoff et al.[13] found the plasma concentration of 17-hydroxycorticosteroids within the normal range when the diabetes was well controlled, but at very high values during diabetic coma. On the other hand, McArthur et al.[14] reported an increased corticoid excretion and a low eosinophil count in the blood of a diabetic patient during insulin reactions. One of our patients developed a moderate insulin reaction during the second trimester. The eosinophil count taken at that time was extremely low.

Vascular complications of diabetes. A relationship between endocrine factors and diabetic vascular disease has been postulated by some authors.[15,16] One of our patients (J.B.) who was studied during three consecutive pregnancies exhibited such complications. In addition to retinopathy, calcification of the left femoral artery was demonstrated on X-ray examination. While in all three pregnancies a tendency toward an increased urinary excretion of corticoids was observed, intercurrent infection and the difficulties encountered in the control of the metabolic disorder render interpretation uncertain.

Maternal weight gain. The average over-all weight gains of pregnant diabetic patients (thirteen to twenty-eight weeks to term: 0.28 kg./wk.) did not differ significantly from those of normal pregnant women (eighteen weeks to term: 0.29 kg./wk.) (table 3). However, the patients almost invariably gained weight at an excessive rate, even in the early stages of pregnancy, when they were at home. Under strict management in the hospital the patients almost invariably lost weight.

TABLE 3

Average weight gain during pregnancy

Patient	Weeks	Kilograms per week
E.S.1	13-36	0.25
E.S.2	13-37	0.25
S.C.	8-36	0.25
B.W.	17-37	0.43
S.H.	10-36	0.24
M.C.	23-36	0.30
P.C.	8-35	0.28
S.R.	10-36	0.32
J.D.	18-36*	0.13*
I.M.	1-32	0.48
J.B.1	16-33	0.39
J.B.2	1-32	0.34
J.B.3	11-37	0.00

*Intrauterine death during the thirty-first week of gestation.

No positive correlation between the degree of weekly weight gain or loss and either fetal survival or excretion of any urinary steroids could be demonstrated.

Toxemia of pregnancy. Six patients developed slight to moderate edema of the lower extremities, and five patients showed traces of albumin in the urine. In only one case (I.M.) were the edema and albuminuria accompanied by a distinct rise in blood pressure, a syndrome which was diagnosed as true preeclampsia. The condition was not affected by a salt-free diet and various diuretics. In none of the six patients with edema was there any significant change in urinary corticoids, nor did the single case of preeclampsia exhibit any change in urinary formaldehydrogenic steroids.

Fetal survival. Of the thirteen pregnancies, eight resulted in fetal survival. Two infants died in utero and three within a day or two after birth. There was no correlation between fetal mortality and the excretion of urinary corticoids. In accordance with the experience of others, the rate of fetal survival diminished, in general, with increasing duration of diabetes.

Crude neutral ketosteroids. The so-called 17-ketosteroid fraction of the urine represents a mixture of steroid metabolites which are principally derived from androgenic hormones. In human pregnancy, their precursors are elaborated by the maternal adrenal cortex and possibly by the ovaries and the fetal adrenals.[18] The extent to which the placenta enters into the production of these hormonal precursors is not known. Normally, the total urinary 17-ketosteroids as determined by the Zimmermann reaction tend to increase during the latter part of pregnancy. This is due principally to 20-ketosteroids such as pregnanolone, which gives a positive reaction with the Zimmermann reagent.

In the thirteen diabetic pregnancies, the crude neutral ketosteroids increased, as in normal pregnancy, during the third trimester (figure 2). This observation is in agreement with that of Nuyens,[19] who found no alteration from the normal in four pregnant diabetics. In ten pregnancies pregnane-3(α) ol, 20-one was isolated from the nonketonic fraction. A progressive increase in the urinary excretion of this compound was found with advancing pregnancy.

Single neutral ketosteroids. Two of the principal components of the crude neutral ketosteroid fraction are androsterone and its isomer etiocholanolone. The precursors of both compounds are mainly androgenic hormones such as testosterone and Δ4-androstenedione, but they may derive partly from C_{21}-steroids such as 17α-hydroxy-progesterone and 17α-hydroxy-11-desoxycorticosterone. It has been assumed that in the nonpregnant female these precursors originate entirely in the adrenal

TABLE 4

Correlation of average urinary corticoid values (in milligrams) per twenty-four hours with degree of control of diabetes during pregnancy

	Weeks of gestation							
Degree of control	5-8	9-12	13-16	17-20	21-24	25-28	29-32	32-37
Excellent Good Good—fair	1.07	0.98	2.34	1.74	1.94	2.30	1.70	2.30
Fair—good Fair Poor	4.22*	2.24*	2.04	2.74*	2.51*	2.40	3.20*	3.00

*Differences between better and poorer control statistically significant ($P<0.01$).

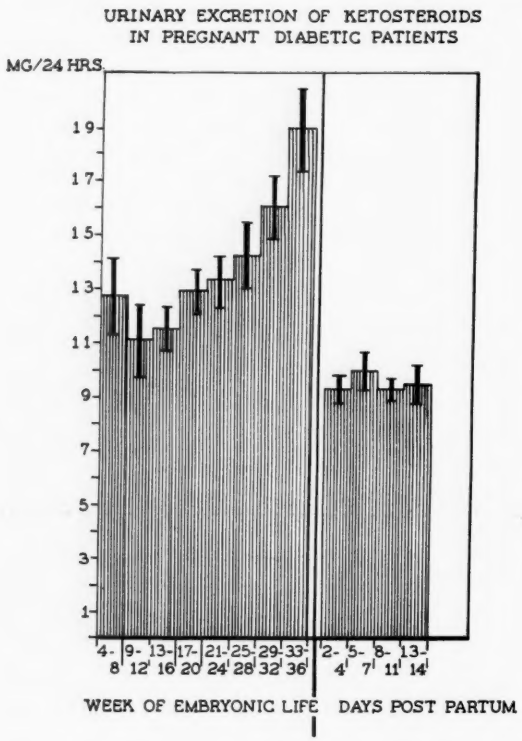


FIGURE 2

cortex. In human pregnancy there is evidence that some of the precursors of urinary androsterone and etiocholanolone may be produced by the ovaries and the fetal adrenal cortex.[18,20,21] Both compounds are excreted in normal amounts during early pregnancy in healthy women. By the eighth month the excretion values are very much reduced, and androsterone may disappear completely from the urine.[6,22] After delivery, the excretion of androsterone and etiocholanolone is restored to normal within a few days.

In nine of the ten diabetic pregnancies in which measurements were made, the urinary excretion of androsterone and etiocholanolone decreased normally until the twenty-ninth week of gestation, but increased significantly thereafter, reaching abnormally high values in the third trimester (figure 3). In the tenth pregnancy there was an abnormal rise of both compounds during the second trimester followed by a decrease to lower levels during the third trimester. No correlation between the abnormal steroidal excretion patterns and clinical observations (insulin requirement, control of diabetes, vascular complications and toxemia of pregnancy) was evident.

The excretion of 11-oxygenated 17-ketosteroids presumably of adrenal cortical origin followed a pattern (figure 3) similar to that of androsterone and etiocholanolone, but the differences between the average values found in pregnant diabetic mothers and those

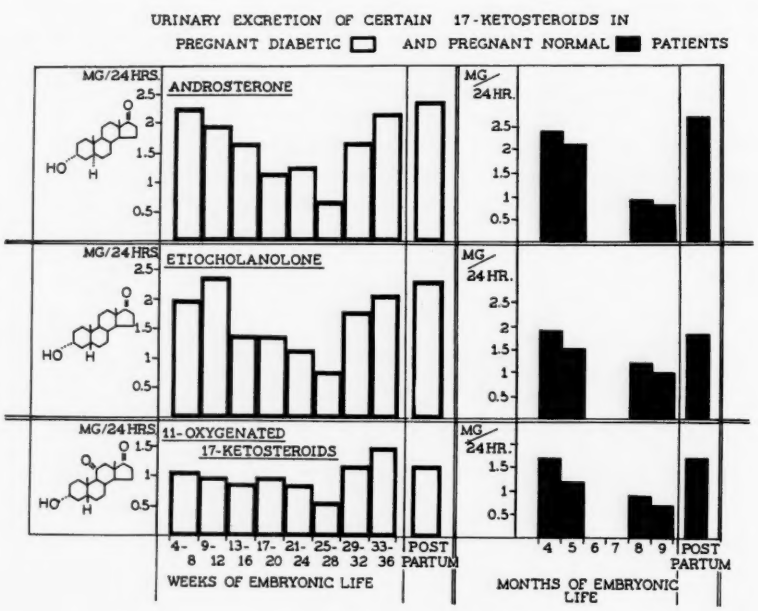
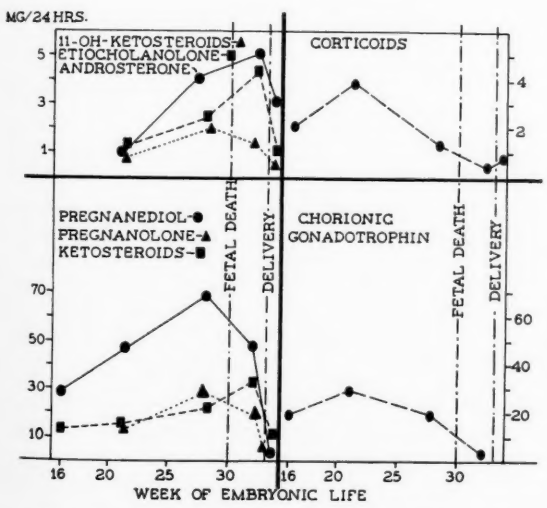


FIGURE 3

of normal pregnant women were not statistically significant.

The increased urinary excretion of metabolites of androgenic hormones late in diabetic pregnancies is of great interest in view of the findings of Bjorklund and Jensen,[23] who reported an excretion of 17-ketosteroids by newborns of diabetic mothers considerably higher than that found in normal controls. These elevated values fell very rapidly and became normal by the fifth day after birth. Thus, the question arises whether the increased urinary excretion of androsterone and etiocholanolone in pregnant diabetics is traceable to activity of the fetal adrenal glands. This explanation seems to be unlikely since in one of our cases (J.D.) a further increase of both compounds was observed in the urine of the mother after the baby had died in utero (figure 4).

On the other hand, the abnormal excretion pattern of androsterone and etiocholanolone in diabetic pregnancies may indicate a disturbance of the endocrine function of the placenta. The normal placenta is apparently capable of converting androgenic steroid hormones into estrogens as shown by in vitro experiments by A. Meyer.[24] It has been demonstrated in our laboratories that in normal pregnancy, C^{14}-labeled testosterone can be converted into tagged estrone. It is possible that the enzyme system responsible for this conversion in the placenta does not function normally during the last trimester of pregnancy in diabetic women. Inhibition of this conversion would result in an accumulation of metabolites of androgenic hormones and a diminution of estrogens. This would be consistent with report of Smith and Smith[2] that estrogens are excreted in decreasing amounts during the last trimester of pregnancies in diabetic mothers.



J.D.-#28 87 28- 23 YRS. OLD- FIRST PREGNANCY- DIABETES MELLITUS SINCE 13 YRS.- INTRAUTERINE FETAL DEATH DURING 30 TH WEEK-

FIGURE 4

The significance of the abnormal excretion of androsterone and etiocholanolone during the last trimester of pregnancy in diabetics is not clear. As true protein anabolizers, androgens produce nitrogen retention and weight increase, the latter to some extent due to the concomitant water and salt retention. Thus, they have some of the properties of a growth hormone. It may be that the excessive growth of fetuses of diabetic mothers is related to an increased activity of the hormonal precursors of androsterone and etiocholanolone.

SUMMARY

1. Excretion patterns of urinary steroids were determined in thirteen pregnancies in diabetic women observed at periodic intervals in the hospital under carefully controlled conditions.

2. Wide fluctuations of the daily corticoid excretion were found during the first and third trimesters, but the average values did not differ significantly from those of healthy pregnant women. During the second trimester, however, the average and, in eleven of thirteen pregnancies, the individual urinary corticoid values were appreciably higher than those of the normal pregnant.

3. A tendency to an increased insulin requirement was observed in all pregnancies. A positive correlation between the insulin dosage and the urinary corticoid values was evident in the majority of the cases, but this correlation was often not apparent during the period immediately preceding delivery.

4. There was a positive correlation between poor control of the diabetic condition during pregnancy and the amounts of corticoids excreted in the urine.

5. Values for the crude urinary neutral ketosteroid fraction, as determined by Zimmermann's reagent, increased normally with advancing pregnancy in all cases.

6. In nine of ten diabetic pregnancies, the urinary excretion of androsterone and etiocholanolone decreased normally until the twenty-ninth week of gestation, increased thereafter, and reached abnormally high values during the third trimester. In the tenth pregnancy, there was an abnormal rise in the excretion of both compounds during the second trimester. No correlation between the abnormal excretion of these steroids and clinical observations was evident. The excretion of 11-oxygenated 17-ketosteroids did not differ significantly from that observed in normal pregnancies.

7. There was no positive correlation between the excretion pattern of corticoids and ketosteroids and the following observations: (1) presence of vascular complications, (2) abnormal maternal weight changes, (3) toxemia of late pregnancy, (4) abnormal fetal weight gain and fetal death, and (5) balances of sodium, chloride, potassium, phosphorus and nitrogen.

SUMMARIO IN INTERLINGUA

Studios Endocrin In Pregnante Feminas Diabetic

1. Le configurationes del excretion urinari de steroides esseva determinate in dece-tres pregnantias in feminas diabetic. Iste determinationes esseva repetite periodicamente. Illos esseva effectuate al hospital sub conditiones cautemente controlate.

2. Extense fluctuationes del diurne excretion corticoide esseva constatate durante le prime e le tertie trimestre, sed le valores medie non differeva significativemente ab illos in gravidas normal. Tamen, durante le secunde trimestre, le excretion urinari de corticoide esseva appreciabilemente plus alte que in gravidas normal, non solmente quanto al valor medie sed etiam quanto al valores individual in dece-un del dece-tres pregnantias studiate.

3. Le tendentia de un augmentate requirimento de insulina esseva notate in omne le pregnantias. Un correlation positive inter le dosage de insulina e le valor del corticoide urinari esseva evidente in le majoritate del casos, sed iste correlation esseva frequentemente non apparente durante le periodo immediatemente ante le parturition.

4. Esseva constatate un correlation positive inter le inadequatia del compensation del condition diabetic durante le pregnantia e le quantitate de corticoides excernite in le urina.

5. Le valores del crude fraction de cetosteroide neutre in le urina, determinate per medio del reagente de Zimmermann, se augmentava normalmente con le avantiamento del pregnantia in omne le casos studiate.

6. In nove ex dece gravidas diabetic, le excretion urinari de androsterona e de etiocholanolona regredeva normalmente usque al vinti-none septimana del gestation, reprogredeva post ille tempore, e attingeva anormalmente alte valores durante le tertie trimestre. Le decime de iste gruppo de gravidas se distingueva per un augmento anormal del excretion de ambe le mentionate compositos durante le secunde trimestre. Nulle correlation esseva evidente inter le excretion anormal e le observationes clinic. Le excretion de 17-cetosteroides 11-oxygenate non differeva significativemente ab illo observate in gravidas normal.

7. Esseva trovate nulle correlation positive inter (de un latere) le configuration del excretion de corticoides e cetosteroides e (del altere latere) ulle del sequente observationes: (1) Presentia de complicationes vascular, (2) anormal alterationes del peso materne, (3) toxemia de pregnantia tardive, (4) anormal augmento de peso fetal e morte del feto, e (5) balancias de natrium, chloruro, kalium, phosphoro, e nitrogeno.

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"Seeing Is Believing and Vice Versa"

Does the conduct of a scientific experiment, once science has advanced beyond the descriptive or natural history stage, require of the investigator anything more than a refined version of the qualities expected of a good witness in court? That is to say, does the conduct of a scientific experiment involve anything more than the careful and detailed observation of a certain set of phenomena by the use of certain instruments? We suppose that the answer is no if the scientist testifies only about those events that he sees directly with his own two eyes. But then you do not have science, only the description of some pieces of equipment. When the report turns to the *results* of an experiment, then something more is involved. Since there is sometimes the temptation to regard an experiment simply as the precise observation of a certain set of phenomena, it may be worth while to remind ourselves of what is also required.

This distinction between ordinary testimony and the report of the results of a scientific experiment was emphasized by Pierre Duhem (1861-1916), a French physicist who is known today primarily for his contributions to the history and philosophy of science. After making this distinction, Duhem went on to examine the meaning of doing an experiment. To understand his conclusions it is not necessary to refer to the very latest developments in experimental inquiry. It will be sufficient for us to consider, as does Duhem, the relationship between what a novice might see upon first entering a physics laboratory stocked with ordinary pieces of equipment and what a physicist might report about his own activities there.

Suppose that our novice upon entering the laboratory spies a tangent galvanometer. He might say that he sees insulated copper wire wrapped around a circular frame in the center of which is suspended a small steel bar. He might also comment that the direction of the bar is indicated by a pointer that can be read against a scale. But our physicist in reporting his own activities probably would make no reference to the small bar or to the direction in which it is pointing. He would say that he is measuring the intensity of the electric current flowing in the copper wire. To bridge the gap, however, between his own report and the testimony of the novice, the physicist might add that he is bringing the reading of the pointer on the scale into a certain formula. But if the physicist continues his efforts at explanation he will find himself giving a course in electromagnetic theory, for the formula is a consequence of the fundamental laws of that discipline and its full understanding requires that one first understand those laws.

All of which means that there is a lot of homework to be done before one becomes a scientist. But to do an experiment requires something more than mere study. There is the simple circumstance that not every event that the novice observes has the set of phenomena under investigation as its cause. To distinguish those events arising from the set of phenomena under investigation from those created or mutilated by the workings of the instrument requires the use of the theory, the novice must also accept it as true. For if he regards the theory as false he will have no basis on which to distinguish between appearance and reality, and if he regards a rival theory as true he will make the distinction in a different way. In addition to the precise observation of a set of phenomena, the conduct of an experiment also requires the interpretation of those phenomena. If seeing is believing, then so also is believing seeing.

Joseph Turner, in *Science*, *128*:3323,
Sept. 5, 1958.

Response of the Blood Glucose to Glucocorticoids in Man

Determination of the Hyperglycemic Potencies of Glucocorticoids

Kelly M. West, M.D., with technical assistance of Dorothy Antonia Wood, Oklahoma City

To estimate the potencies of new glucocorticoids, experiments are usually conducted to measure the intensity of several of the physiologic effects produced by the drug. The effect of a glucocorticoid on carbohydrate metabolism is usually measured by determining the degree to which it is capable of promoting the deposition of glycogen in the livers of experimental animals. Although there have been disparities in some instances, the "liver glycogen" potencies of glucocorticoids in animals have correlated in a general way with their potencies in other respects such as inhibition of inflammation, inhibition of secretion of adrenocorticotrophic hormone and depression of the number of circulating eosinophiles.

On the other hand, disparities between the results of experiments in animals and man[1,2] have necessitated the development of better methods of measuring the hyperglycemic potencies of glucocorticoids in man. In testing the potencies of these drugs in man, their effects on carbohydrate metabolism have not usually been used as an index of potency. This is probably because there are certain obstacles which have been encountered in trying to determine quantitatively the hyperglycemic effects of glucocorticoids in human subjects. The measurement of liver glycogen is, of course, not generally feasible, and the hyperglycemic effects of these drugs vary greatly from individual to individual.[3,4] Furthermore, under some experimental conditions, no appreciable effect was produced on glucose tolerance by the administration of even large doses of glucocorticoids.[5,7] Lastly, under certain circumstances no effect on the fasting blood glucose had been noted after the administration of these drugs.[6,7]

On the other hand, it has been shown previously that under the proper circumstances impairment of glucose tolerance could be produced regularly by the administration of two doses of several different glucocorticoids,[8] and furthermore, that the degree of impairment produced was well correlated with the potency of the drug being tested.[8] While there were substantial variations in the hyperglycemic responses from subject to subject, the responses of each individual, from day to day, to equivalent doses of glucocorticoids were fairly consistent in magnitude.

The relatively predictable hyperglycemic effect on oral glucose tolerance tests produced by several glucocorticoids under the circumstances of our previous experiments were encouraging, so it was decided to explore further some of the factors which influence response of the blood glucose to glucocorticoids by testing the effects of these drugs on intravenous glucose tolerance and on the fasting blood glucose. Another purpose of the experiments was to evaluate certain methods of establishing the hyperglycemic potency of a glucocorticoid.

METHODS

The glucocorticoids were administered by mouth. The blood glucose determinations were done by the method of Nelson[9] on venous blood. The subjects were healthy adults varying in age from twenty to fifty-six years, the vast majority of whom were between twenty and thirty-five years of age. There were approximately equal numbers of males and females. The subjects were not placed on formal diets during testing but were questioned concerning their diets and no individuals were tested who had in any way restricted their diets previous to or during the experiments. At least forty-eight hours elapsed between each test with a drug. The effect of the glucocorticoid on the fasting blood glucose was estimated by comparing the level of the blood glucose after the drug with the fasting blood glucose at the same hour on a previous control day or days. (The average intra-individual variability of the control fasting blood glucose values from day to day, based on triplicate tests in ten randomly chosen individuals, was 4.3 mg. per cent

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with a standard deviation of 3.2 mg. per cent.)

The effect on glucose tolerance was measured by comparing the blood glucose one hour after an intravenous glucose load of 25 gm. on a control day with the one-hour blood glucose after the drug. The glucose was administered in two to four minutes as 50 ml. of 50 per cent glucose and the test timed from the beginning of the injection. (The one-hour blood glucose is, of course, not the best possible index of glucose tolerance. However, since it is possible to identify with a few tests a small difference in glucocorticoid potency using this simple method of estimating glucose tolerance, it does seem adequate for this purpose.)

FACTORS WHICH INFLUENCE THE RESPONSE OF THE BLOOD GLUCOSE TO GLUCOCORTICOIDS

Effect of glucose loading. It is apparent from examining the data in table 1 that glucose loading increases the sensitivity of the blood glucose to the administration of prednisolone. The administration of 10 mg. of prednisolone four hours and eleven hours before an intravenous glucose load of 25 gm. produced an elevation of the one-hour blood glucose which averaged 41 mg. per cent over control values. On the other hand, the elevations of the fasting blood glucose averaged only 18 mg. per cent after the same treatment. The substantial variations in the responses among individuals are apparent.

Sensitivity of fasting blood glucose to glucocorticoids. Although the fasting blood glucose is not very responsive to the administration of glucocorticoids it is sufficiently responsive under certain circumstances to enable one to estimate the hyperglycemic potency of a glucocorticoid without including glucose loading as part of the test. It was found, for example, that a small but consistent effect was produced on the fasting blood glucose when 15 mg. of prednisolone was administered four hours prior to the glucose determination (table 2). Table 2 also shows that 5 mg. produced a less consistent effect which was not statistically significant. As will be indicated in the text immediately below, the fasting blood glucose is more sensitive to the glucocorticoid if the drug is administered eight hours before the determination.

TABLE 1

Effect of prednisolone on fasting blood glucose and on glucose tolerance

	Elevation* of blood glucose in mg. per cent produced by two 10 mg. doses of prednisolone†	
Subject No.	Fasting glucose	One-hour glucose
1	17	20
2	19	67
3	15	29
4	29	69
5	11	51
6	6	—2
7	35	44
8	29	56
9	11	20
10	8	51
Mean	18	41

*The elevation of the fasting blood glucose was determined by comparing the fasting blood glucose at 10 a.m. on a control day with the fasting blood glucose at that time after administration of the drug. The elevation of the blood glucose one hour after an intravenous glucose load produced by the drug was determined by comparing the one-hour blood glucose on a control day with the one-hour glucose on the day the drug was administered.

†Administered orally four hours and eleven hours before the fasting blood glucose and the intravenous glucose.

TABLE 2

Effect of two different doses of prednisolone on fasting blood glucose

	Elevation* of fasting blood glucose in mg. per cent four hours after prednisolone	
Subject No.	5 mg.	15 mg.
1	8	11
2	9	8
3	18	11
4	12	11
5	13	21
6	0	10
7	—3	9
8	—10	4
9	—4	2
10	3	6
11	10	6
12	—2	11
Mean	4.5	9

*Over the mean of two fasting blood glucose values on control days at the same hour (10 a.m.). The hyperglycemic effect of 15 mg. was statistically significant ($p<.01$) and that of 5 mg. was not. When 5 mg. prednisolone was administered eight hours before a fasting blood glucose it produced a significant effect (see text).

The delayed hyperglycemic effect of glucocorticoids. It will be apparent from examining table 3 that when 40 mg. hydrocortisone was administered eight hours before a blood glucose determination a greater effect was produced than that produced when this hormone was administered four hours before the fasting blood glucose determination. This phenomenon is of particular interest because it has been shown that an insignificant amount of the hormone is present in the blood eight hours after it is administered orally in doses even larger than these.[10]

It is also of interest in this connection that when this same dose of hydrocortisone was administered by mouth to ten subjects, in this laboratory, the eosinopenic effect was significantly greater at four hours than at eight

TABLE 3

The "delayed" hyperglycemic effect of hydrocortisone

Subject No.	Fasting blood glucose in mg. per cent at 8:00 a.m.	
	Four hours after 40 mg. hydrocortisone	Eight hours after 40 mg. hydrocortisone
1	92	94
2	98	115
3	75	77
4	70	73
5	73	82
6	85	100
7	92	87
8	80	87
9	80	80
10	96	109
Mean	84	90
	$p<.03$	

The order of testing was randomized.

hours ($p<.01$).

Further evidence that the hyperglycemic action of a dose of a glucocorticoid is prolonged has been accumulated in this laboratory. Subjects were given 10 mg. of prednisolone four hours before a fasting blood glucose. On another occasion the test was repeated adding a second dose eleven hours before the blood glucose determination. The results of this experiment are recorded in table 4. It may be noted that the blood glucose values were significantly higher ($p<.01$) when this dose was included even though it was administered eleven hours before the test. These results may be compared with those of Duncan,[7] who found no elevation of the fasting blood glucose and no impairment of intravenous glucose tolerance two hours after 200 mg. of cortisone.

TABLE 4

Hyperglycemic effect of prednisolone administered eleven hours before a fasting blood glucose determination

Subject No.	Elevation* of fasting blood glucose in mg. per cent after prednisolone	
	Four hours after one dose of 10 mg.	After two 10 mg. doses (four and eleven hours before determination)
1	0	17
2	24	30
3	12	19
4	15	15
5	3	15
6	9	29
7	15	15
8	0	6
9	13	35
10	12	29
Mean	10	21
	$p<.01$	

*Over a control value on a previous day at the same hour (10 a.m.).

It seems likely that the latter negative results were due to the fact that the glucose tolerance tests were done before the onset of the hyperglycemic action of cortisone. In this laboratory only three intravenous glucose tolerance tests have been made as early as two hours after a single 10 mg. dose of prednisolone. In each instance the drug produced no significant effect.

It may be noted that when 5 mg. of prednisolone was administered four hours before fasting blood glucose determination, the small effect was not statistically significant (see table 2). On the other hand, when 5 mg. of prednisolone was administered to ten additional subjects eight hours before a fasting blood glucose a highly significant effect was produced ($p<.01$). On still another occasion, ten subjects responded significantly under these circumstances (see figure 1).

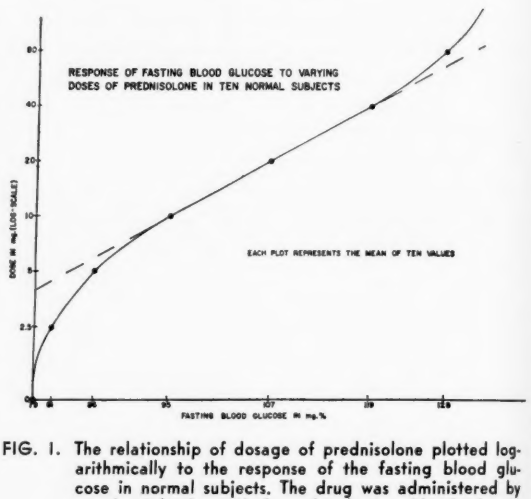

FIG. 1. The relationship of dosage of prednisolone plotted logarithmically to the response of the fasting blood glucose in normal subjects. The drug was administered by mouth eight hours before the fasting blood glucose determination. The order in which the various doses were administered was randomized. The broken line shows that as the dose was raised from 10 mg. through 40 mg. the magnitude of the responses obtained was directly related to the dosage logarithmically expressed.

That the peak of hyperglycemic action of a 40 mg. dose of hydrocortisone occurred earlier than twelve hours after administration by mouth was shown when ten subjects exhibited significantly greater blood glucose values at 8:00 a.m. eight hours after the drug on one occasion than at 8:00 a.m. twelve hours after the drug on another occasion ($p<.02$). The order of the tests was randomized.

Our experience concerning the delay in the onset of hyperglycemic action of glucocorticoids is similar to the experience of Glenn, Stafford and Bowman,[11] who found recently that the maximum effect on the production of

liver glycogen in rats occurred about eight hours after the administration of hydrocortisone subcutaneously, at a time when there was an insignificant amount of the hormone in the blood. They accumulated additional data which suggested that the deposition of liver glycogen might be secondary to a peripheral "nonhepatic" effect of the hormone.

It is apparent that the data presented in this paper do not permit conclusions concerning the precise time at which the peak hyperglycemic action occurs after a glucocorticoid is administered orally. This point is being explored in this laboratory. Our preliminary studies suggest that the peak action occurs six to eight hours following administration of prednisolone and of hydrocortisone by mouth, although the data are not adequate as yet to permit a final conclusion. Furthermore, it is not known whether the peak of action occurs at the same hour irrespective of the dosage used.

The effect of heredity on the hyperglycemic response to glucocorticoids. Fajans and Conn[4] found that nondiabetic persons with a family history of diabetes exhibited greater hyperglycemic responses to cortisone than did persons with no family history of diabetes. In this laboratory it was found that nondiabetic subjects with a family history of diabetes exhibited significantly greater hyperglycemic responses to prednisolone and prednisone than a control group.[3] Furthermore, the hyperglycemic responses of identical twins were found to be strikingly familiar. For example, of twenty subjects the two who showed the greatest responses to glucocorticoids were twins and the two who showed the smallest responses were also twins.

The correlation between potency and hyperglycemic response. Table 5 shows the fasting blood glucose values eight hours after the administration of 10 mg. of prednisolone and after 15 mg. The order of testing was randomized. It is of interest that this relatively small difference in potency was established after testing only ten subjects since p was less than .02. These results suggested that this particular method was a simple but sensitive way of establishing the hyperglycemic potency of a dose of glucocorticoid.

The relationship between dosage and the intensity of hyperglycemic response was explored further by using another method. Ten subjects were given doses of prednisolone eleven hours before and four hours before an intravenous glucose tolerance test. Their blood glucose values one hour after a load of glucose 25 gm. were compared with their one-hour blood glucose values at the same hour on a control day. The elevations of one-hour blood glucose produced by the 10 mg. doses, and by the 15 mg. doses of prednisolone administered in alternating order, are recorded in table 6. Since their responses were significantly greater after the 15 mg. doses ($p<.01$) it would appear that this particular method is also capable of identifying relatively small differences in potency.

The relationship between the size of the dose administered and the response of the fasting blood glucose was more completely explored by testing the responses of ten normal subjects, eight hours after seven different

TABLE 5

The hyperglycemic response to 10 mg. and to 15 mg. of prednisolone

	Fasting blood glucose in mg. per cent eight hours after prednisolone		
Subject No.	Control	10 mg.	15 mg.
1	79	87	92
2	83	83	87
3	80	87	92
4	69	79	96
5	77	87	90
6	69	83	96
7	73	79	79
8	83	96	92
9	79	87	98
10	80	83	95
Mean	77	85	92

10 mg. produced a significant effect ($p<.01$).
15 mg. produced an effect significantly greater than 10 mg. ($p<.02$).

TABLE 6

The response of the one-hour intravenous tolerance test to prednisolone

	Elevation* of one-hour blood glucose produced by prednisolone in mg. per cent	
Subject No.	Two 10 mg. doses†	Two 15 mg. doses
1	20	27
2	67	81
3	29	63
4	69	129
5	51	64
6	—2	25
7	44	62
8	56	76
9	20	32
10	51	71
Mean	41	63
	$p<.01$	

*The elevation of the blood glucose produced by the drug one hour after an intravenous glucose load of 25 gm. was determined by comparing the one-hour blood glucose on a control day with the one-hour blood glucose on the day the drug was administered.
†Administered orally four hours and eleven hours before the glucose load.

doses of prednisolone. The results of these experiments are recorded in figure 1. The responses to doses of 2.5, 5, 10, 20, 40 and 80 mg. were plotted graphically (dosage logarithmically plotted). It may be noted that when these plots were joined an S-shaped curve was formed, suggesting that very low doses of the order of 2.5 mg. produce little if any response, and that when doses of 80 mg. were administered, the point of maximum response was being approached. It is of interest that the plots of the responses to doses of from 5 mg. to 40 mg. fall in a line which is relatively straight and that the line joining the plots of doses of 10 mg. to 40 mg. is quite straight. Apparently, in this range of dosage, there is an excellent correlation between the log of the dose and the response. It is of further interest that even though only ten subjects were used, doubling the dose invariably resulted in responses which were greater to a statistically significant degree since p was less than .02 in each instance.

EXPERIMENTAL DESIGN IN TESTING THE HYPERGLYCEMIC POTENCIES OF GLUCOCORTICOIDS

Since there is a good correlation between the size of the administered dose and the hyperglycemic responses under certain conditions described above, it has been possible to design methods of establishing the hyperglycemic potency of a glucocorticoid of unknown potency.

It would appear that the following points are of importance in determining the hyperglycemic potency of a glucocorticoid: (1) The response of the blood glucose to the glucocorticoid must be compared with the response in a control situation *in the same individual.* The interindividual variation in response is so great (see table 1) that a much larger number of tests would have to be made in order to demonstrate statistically significant differences in potency between two drugs if a paired control system was not being used. Such data are analyzed using a paired control t test. (2) If a very small amount of a drug is available, glucose loading could be included as part of the control test, and the test with the drug in order to "sensitize" the blood glucose to smaller doses of the drug or drugs. (3) Allowance must be made for the delayed onset of the hyperglycemic effect (see table 3). (4) Randomization of the order of testing is desirable when the potencies of several doses of drugs are being compared in series, and at least forty-eight hours should elapse between tests. Although we have noticed no cumulative effect in testing a small series of single doses of drugs in the same subject under the conditions described under Methods, there is a possibility that under certain conditions previous tests might affect the results of a subsequent test. Furthermore, there is a possibility that technical factors in the determinations of glucose might produce slightly different results from day to day on samples containing identical amounts of glucose. (5) Selection of appropriate dosages. A dose must be used as a standard which will produce responses consistently, but this dose should not produce a maximum response. The dosage required to elevate the blood glucose regularly depends upon the type of test carried out. It is apparent from the data in figure 1 that a single dose of a glucocorticoid equivalent in potency to 5 mg. of prednisolone when administered by mouth eight hours before the fasting blood glucose determination will produce a hyperglycemic effect regularly, and that the administration of a dose which is substantially more potent will result in substantially greater responses. In the dosage range of 5 mg. to 80 mg. the response expressed arithmetically was very closely correlated with the dose logarithmically expressed.

During preliminary studies several methods of estimating hyperglycemic potency of glucocorticoids were used. It appears that the most satisfactory is the simple method described above in which hyperglycemic potency is measured by comparing the fasting blood glucose eight hours after the drug is administered orally with the fasting blood glucose at the same hour on a control day. The subjects are instructed not to eat after 7:00 p.m. on either day. Under these conditions a standard dose of glucocorticoid of known potency has been used (usually 5-20 mg. of prednisolone or 40-80 mg. of hydrocortisone) with which the potency of the new drug is compared. An example of such an experiment will be cited below.

TESTING THE HYPERGLYCEMIC POTENCY OF A GLUCOCORTICOID OF UNKNOWN POTENCY

The principles discussed above have been applied in testing the hyperglycemic potencies of a series of glucocorticoids. These results have been the subject of a separate report.[1] The results of an experiment designed to establish the hyperglycemic potency of 6-methyl prednisolone (Medrol) are reported here in order that they may serve as example of the applicability of the method.

On three separate occasions single doses of 15 mg. 6-methyl prednisolone, 15 mg. prednisolone, and 22.5 mg. of prednisolone were administered by mouth in random order to each of ten healthy subjects, at intervals of not less than two days. Eight hours after the drug was administered a fasting blood glucose was determined at 8:00 a.m. The results, which are recorded in table 7, indicate that it was possible to identify clearly the small difference between the potency of the 15 mg. doses of prednisolone and the 22.5 mg. doses of prednisolone

TABLE 7

The relative hyperglycemic potencies of prednisolone and 6-methyl prednisolone

	Fasting blood glucose values in mg. per cent eight hours after drug		
Subject No.	Prednisolone 15 mg.	6-methyl Prednisolone 15 mg.	Prednisolone 22.5 mg.
1	105	87	107
2	95	85	95
3	87	83	95
4	94	83	102
5	96	94	94
6	85	83	85
7	98	87	105
8	100	82	92
9	96	95	110
10	100	110	114
Mean	96	89	100
		$p<.04$	$p<.01$

The order of testing the drugs was randomized.

This table contains data previously reported (West, K. M.: "The Eosinopenic and Hyperglycemic Potencies of Glucocorticoids in Man." *Metabolism,* July (Part II), 1958). The data are reproduced by permission of *Metabolism.*

($p<.01$). This again confirmed the sensitivity of the method. Medrol was found to be more potent than prednisolone milligram for milligram ($p<.04$). The mean response to 22.5 mg. prednisolone was greater than to 15 mg. Medrol. However, this difference is not statistically significant. These data suggest that the hyperglycemic potency of Medrol is about 30 per cent greater than prednisolone.

In testing the potencies of the new corticoids, we have found that, in general, six to twelve subjects must be tested in this manner in order to distinguish clearly a difference in potency between a control dose and a dose which is twice as potent or half as potent. When doses which differ only as much as 50 per cent are tested, ten to thirty pairs of tests are usually required before statistically significant differences are apparent.

DISCUSSION

It may be deduced from the data above concerning the hyperglycemic time-action characteristics of hydrocortisone and prednisolone that their physiologic time-actions in this respect do not coincide with the curves described by their blood levels. It may be that the time-action curves in other physiologic respects of glucocorticoids do not reflect directly their rates of disappearance from the blood. For example, Ely and associates[10] found that the half time of a dose of intravenously administered hydrocortisone averaged ninety minutes as compared to an average half time for prednisolone of 192 minutes. On the other hand, the author found that the eosinopenic time-action curves of these two drugs were indistinguishable when doses of similar potency were administered (20 mg. hydrocortisone and 5 mg. prednisolone).[1] It has been found in this laboratory that the following glucocorticoids have very similar eosinopenic time-action curves when administered orally in doses of similar potency: hydrocortisone, prednisolone, 6-methyl, prednisolone, 9-alpha-fluorohydrocortisone, and triamcinolone.[1]

It appears that the biologic time-action curve of a dose of a glucocorticoid depends on the potency of the dose which is in turn dependent on the size of the dose and the molecular configuration of the glucocorticoid. Thus, milligram for milligram, highly potent glucocorticoids such as prednisolone and 9-alpha-fluorohydrocortisone are "longer acting" than less potent glucocorticoids such as hydrocortisone, but when the more potent drug is administered in doses equivalent in potency to a dose of the less potent glucocorticoid it is not longer acting.

On the other hand, it is possible that the hyperglycemic time-action curves of different glucocorticoids are not similar, even when they are administered in doses of comparable potency. Although there is very little direct evidence available concerning this point, our experience to date suggests that the hyperglycemic time-action curves are similar when doses of comparable potency of different glucocorticoids are administered. For example, it was found that 10 mg. of prednisolone was equivalent in hyperglycemic potency to 40 mg. of hydrocortisone, irrespective of whether the doses of the drugs were administered 4, 8, 11, or 11½ hours before the blood glucose determinations,[1-3] and the relative hyperglycemic potencies of prednisolone and 6-methyl, prednisolone were in the same ratio at four hours and eight hours after they were administered.[1]

Since, under the conditions described above, there was a very good correlation between the potency of a dose of a glucocorticoid and the magnitude of the hyperglycemic response, and since it was possible to identify relatively small differences in potency by testing only a few subjects, it has become possible to estimate in human beings quickly and easily the hyperglycemic potency of a new glucocorticoid.

The hyperglycemic potencies of several of the newer glucocorticoids have been established recently in this laboratory using the methods described in this paper. The details of those experiments are reported elsewhere.[1] It is of interest, however, that the hyperglycemic potencies in man of all the glucocorticoids tested in this laboratory have been closely correlated with their anti-inflammatory potencies in man. This suggests that one

may deduce the anti-inflammatory potency of a glucocorticoid by testing its hyperglycemic potency in a few subjects using the simple technics described in this paper.

SUMMARY

Although the fasting blood glucose in man is not as responsive to the administration of glucocorticoids as is the blood glucose in the course of the intravenous glucose tolerance test, small elevations in fasting blood glucose were consistently produced under certain conditions by a single dose of a glucocorticoid. The magnitude of the hyperglycemia was variable from subject to subject but in each subject the magnitude of response produced by a single dose correlated well with the potency of the dose administered at certain levels of dosage.

Evidence is presented suggesting that the peak of hyperglycemic action of hydrocortisone occurs about six to eight hours after a dose is administered orally at a time when the peak of eosinopenic action has passed.

A simple method of determining the hyperglycemic potency of a glucocorticoid is described.

SUMMARIO IN INTERLINGUA

Factores Que Affice Le Responsa Del Glucosa De Sanguine Al Administration De Glucocorticoides In Humanos: Le Determination Del Potentias Hyperglycemic De Glucocorticoides

Ben que le glucosa del sanguine in humanos in stato jejun es minus responsive al administration de glucocorticoides que in le test de toleration pro glucosa intravenose, micre elevationes del glucosa sanguinee in stato jejun esseva effectuate regularmente sub certe conditiones per un dose unic de glucocorticoide. Le magnitude del hyperglycemia esseva variabile ab un subjecto al altere, sed in omne individuo, le magnitude del responsa producite per un dose unic se monstrava ben correlationate con le potentia del dose administrate a certe nivellos de dosage.

Es presentate datos a provar que le maximo del action hyperglycemic de hydrocortisona occurre circa sex a octo horas post le administration de un dose oral, quando le maximo del action eosinopenic ha passate.

Es describite un simple methodo pro determinar le potentia hyperglycemic de un glucocorticoide.

ACKNOWLEDGMENT

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Insulin-like Activity of Normal and Diabetic Human Serum

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In 1957, Martin and Renold[1] devised a method of insulin assay utilizing glucose uptake by Wistar rat adipose tissue, and have reported sensitivity to as little as 10 micro-units/ml. of insulin.[2,3] For the past nine months, this method has been under study at the University of Southern California Medical School, and its great sensitivity to insulin confirmed.[4,5]

METHODS AND MATERIALS

Epididymal adipose tissue from Wistar male rats weighing 100-250 gm. was employed. Segments of tissue were quickly excised, weighed,* immersed in Krebs-bicarbonate medium[6] containing glucose, and incubated in a Dubnoff shaker.† Weight of individual tissue segments was usually in the 40-70 mg. range. Incubation proceeded for a period of two to four hours at a temperature of 36.5° C. under 95 per cent O_2 — 5 per cent CO_2. Two to 2.5 ml. of incubation medium was used in which the appropriate control and test substances were incorporated. The glucose concentration was usually 1-1.5 mg./ml. With each set of noninsulin controls, of insulin determinations, and of experimental sera, a "blank" was incorporated consisting of ingredients identical to the material being incubated with adipose tissue, but containing no such tissue. Glucose determinations were performed simultaneously of the "blanks" and of the media containing adipose tissue following incubation. The difference between the "blank" and experimental glucose levels, expressed as total milligrams of glucose, denoted disappearance of glucose from the incubation medium, and was assumed to be equivalent to glucose uptake by the adipose tissue. Correction was made for the tissue weight and the calculation expressed mg. glucose per gm. of adipose tissue. Glucose concentrations were determined by the Somogyi-Nelson method.[7]

Various bloods were collected, permitted to clot, and the sera immediately separated, or the clotted blood was refrigerated and the sera later separated. Normal sera were obtained one quarter to two hours postprandially from young adults aged approximately twenty to thirty-five years. The diabetic coma sera were secured at time of admission and at various intervals during the course of therapy.

Each set of determinations usually included the appropriate "blanks," controls consisting of tissue in Krebs-bicarbonate glucose containing 0.2 per cent serum albumin,* identical media containing various concentrations of insulin,† and the various human sera being studied, diluted one tenth in Krebs-bicarbonate glucose.

RESULTS

It was found that the most satisfactory random distribution was obtained if glucose uptake values (mg. glucose/gm. adipose tissue) were converted into the corresponding $\log_{10}$ value. A satisfactory relationship ($r = 0.54$) was noted between increase in glucose uptake and amount of insulin in the 1 to 100 micro-unit per ml. dose range (table 1). In this report, significance of mean uptake values was estimated and probabilities ascertained with Student's "t" method. Detailed studies, to be reported separately, have been made of the various factors which conceivably alter the glucose uptake, including original concentration of glucose in the medium, weight of individual tissue segments, length of incubation time, concentration of serum protein, state of nutrition of the experimental animal, and total weight of the rat.[5] One variable of definite importance, according

*August Sauter Balance.

†Dubnoff Metabolic Shaking Incubator, Precision Scientific Co.

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*Normal Serum Albumin (Human), salt-poor, USP, Hyland Laboratories.

†Crystalline insulin was obtained from the U.S. Pharmacopeia through the courtesy of Dr. Lloyd Miller.

TABLE 1

Relationship of insulin concentration to glucose uptake

Insulin conc. micro-units/ml.	Number of determinations	Mean glucose uptake* mg. glucose gm. adipose tissue	r†
0 (0.2 per cent albumin control)	38	4.32	
1	24	5.05	
10	38	6.98	0.54
100	34	13.05	
1,000	14	13.01	

*Geometric mean glucose uptake as mg. glucose/gm. adipose tissue.
†Correlation coefficient.

to these studies, is the weight of the rat. Increase in glucose uptake appears to be associated with diminution of animal weight. Adipose tissue from rats weighing considerably more than 300 gm. quite frequently demonstrated no appreciable increase in glucose uptake with doses of insulin as high as 100 micro-units/ml.

The sensitivity and reproducibility of this system in relation to various categories of human serum was determined by calculating the difference between the mean glucose uptakes of these sera and the corresponding nonserum control uptakes. For example, in a single run, normal or diabetic sera diluted one tenth in Krebs-bicarbonate-glucose, and nonserum Krebs-bicarbonate-glucose controls* would contain adipose tissue from a single animal. The difference between glucose uptakes of these sera and their corresponding controls, performed simultaneously with tissue from the same rat, would be calculated. The same procedure was carried out with pretherapy diabetic coma sera and nonserum controls. The mean uptakes, calculated as difference in glucose uptake compared with the corresponding nonserum control, permitted a single mean value for each individual serum to be designated. Single mean values were obtained for each post-treatment diabetic coma serum specimen by calculating mean difference in glucose uptake as compared with the mean glucose uptake of precoma serum from the same case. Post-therapy diabetic coma sera were compared with the pretherapy diabetic coma sera from the same case. Determinations, however, were usually not simultaneous. The mean increase in glucose uptake was determined for each class of serum, normal, pretherapy diabetic coma, and post-treatment diabetic coma, and the statistical significance was calculated (table 2, figure 1). There was a significant increase in glucose uptake for the normal postprandial sera as compared with corresponding controls ($P>0.01$, <0.02). The pretherapy diabetic coma value showed no significant increase ($P>0.4$). The mean increase in glucose uptake of post-therapy diabetic coma sera, as compared with pretherapy diabetic sera, was significantly increased ($P<0.001$).

*0.2 per cent human serum albumin incorporated in these controls.

TABLE 2

Comparison of insulin-like activities of normal pretreatment diabetic coma and post-therapy diabetic coma sera

Category of serum*	Number of sera	Mean alteration of glucose uptake† (mg. per cent glucose/gm. fat-$\log_{10}$)	±S.E.	t	P
Normal	15	+.127	.046	2.75	>.01,<.02
Pre-treatment diabetic coma	8	+.043	.052	0.83	>.4
Post-treatment diabetic coma	35	+.168	.034	4.94	<.001

*Sera diluted one tenth in Krebs-bicarbonate and glucose.
†Glucose uptake alteration is calculated as geometric mean of change in glucose uptake of individual normal and pretreatment diabetic coma sera as compared with corresponding control glucose uptakes. Controls consisted of 0.2 per cent albumin in Krebs-bicarbonate glucose assayed, at same time, with adipose tissue from same animals as test sera. Post-treatment diabetic coma serum values represented difference between mean glucose uptakes of post-treatment and pretherapy sera of each coma case.

As an alternative approach, the individual mean glucose uptakes have been recorded for the various normal individuals. These are expressed as absolute glucose uptakes. The differences between glucose uptakes of sera and corresponding controls are not presented, but a separate calculation of mean control glucose uptake, composed of all control determinations performed in association with normal sera, is given. All serum specimens were obtained one quarter to two hours postprandially (table 3, figure 2). It is probably not justifiable to determine the exact significance of each normal serum value in view of the large standard error associated with the control uptakes. Apparently significant differences between glucose uptakes of individual sera could be in part explained by the variation of the method or, possibly, by error in sampling. Nevertheless, there appears to be considerable inherent individual variation of insulin-like activity in normal serum, which may be associated with various physiological factors.

Two to ten serial serum specimens were obtained from

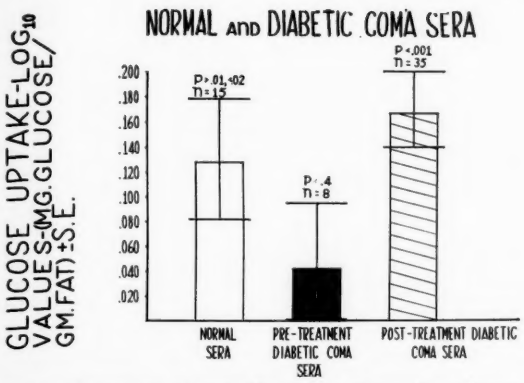

FIG. 1. Comparison of insulin-like activity of normal, pretherapy diabetic coma, and post-treatment diabetic coma sera. Insulin-like activities computed by calculating mean difference between glucose uptakes of individual normal sera and of corresponding control glucose uptakes obtained simultaneously. Mean difference calculated for individual pretherapy diabetic coma sera in identical fashion. Insulin-like activity of post-treatment diabetic coma sera computed by calculating mean differences between glucose uptakes at post-treatment and pretherapy sera from same coma subject. Mean values of each category of normal, pretherapy and post-treatment coma sera calculated from these individual serum glucose uptakes. Glucose uptakes represented as $\log_{10}$ mg. glucose/gm. adipose tissue. Bars indicate standard error of geometric mean. Numbers above bars represent number of sera. P calculated by "t" test.

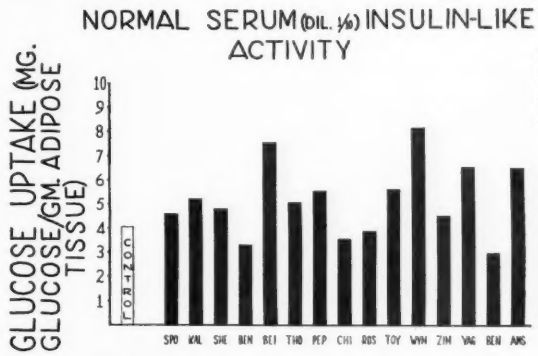

FIG. 2. Insulin-like activity of normal postprandial sera. Sera obtained ¼-2 hours postprandially. Glucose uptake calculated as antilog of geometric mean. (Control category composed of all control determinations performed coincident with normal serum glucose uptake determinations.)

eight diabetic coma cases and, as explained above, mean insulin-like activity was significantly diminished in the initial pretherapy specimens. A study of the individual coma serum specimens utilizing absolute glucose uptakes, rather than differences between controls and corresponding serum specimens, indicates considerable individual variation exists (table 4, figures 3, 4, 5). In part this may be attributable, as with normal sera, to the variation of the assay method and to possible sample error. However, the glucose uptake values also reflect, probably, intrinsic serum insulin-like activity to some degree.

One coma case (Som.) demonstrated substantial in-

TABLE 3

Insulin-like activity of normal sera (diluted 1/10)

Subject	Number of determinations	Time of collection postprandial (hours)*	Serum glucose (mg. per cent)	Mean glucose uptake† (mg. per cent glucose per gm. adipose tissue)	90 per cent confidence limits
Control‡	17			4.08	3.38-4.92
Spo (M)	4	2	87	4.73	3.10-7.23
Kal (M)	4	2	65	5.22	2.52-10.80
She (F)	4	2	71	4.90	3.90-6.14
Ben (F)	4	2	43	3.33	2.37-4.63
Bei (M)	4	¼	108	7.75	4.84-12.70
Tho (F)	4	2	48	5.15	3.64-7.27
Pep (F)	3	2	52	5.56	2.97-10.40
Chi (M)	4	½	—	3.59	2.04-6.32
Ros (M)	6	1	91	3.95	2.10-7.42
Toy (M)	6	¾	—	5.70	4.13-7.87
Wym (M)	4	1	106	8.24	5.79-11.70
Zim (M)	4	½	105	4.54	3.78-5.46
Yag (F)	5	1½	—	6.25	5.01-8.49
Ben (F)	8	¼	169	3.03	2.00-4.60
Ams (M)	4	1½	—	6.52	5.71-7.62

*Interval of time between termination of eating and collection of serum.
†Geometric mean.
‡Control category includes all glucose uptakes of 0.2 per cent albumin Krebs-bicarbonate determined coincident with normal serum glucose uptakes.

TABLE 4

Insulin-like activity of diabetic coma sera preceding therapy and in the course of treatment (diluted 1/10)

Subject	Serum number	Number of determinations per serum	Mean glucose uptake*	90 per cent confidence limits	Interval of time following commencement of therapy (hours)	Cumulative insulin dosage (units)	Blood glucose level (mg. per cent)	Comment
Kal	*Control‡*	*18*	*3.13*	*1.91-5.12*				
64,	1	4	2.92	1.20-7.14	0	0	1,438	CO_2=5
Fe	2	11	6.62	5.55-7.91	3	400	1,214	CO_2=5
	3	4	3.76	1.99-7.11	6	800	904	CO_2=9.5
	4	8	9.23	7.28-11.70	8	1,200	795	CO_2=14
	5	7	7.15	5.53-9.23	11	1,500	—	
	6	11	5.25	3.12-8.83	14	1,800	358	CO_2=27
	7	4	5.21	3.03-8.95	17	1,840	368	
	8	4	5.70	3.78-8.59	20	1,920	301	CO_2=28
	9	7	2.41	1.46-3.97	24	1,920	238	Water
	10	4	7.18	5.93-8.69	30	1,960		Intoxication
Som	*Control*	*11*	*4.47*	*3.25-6.14*				
26,	1	4	7.53	5.50-10.33	0	0	740	
Ma	2	8	7.69	5.28-1.19	1½	200	—	
	3	12	4.92	3.66-6.61	7	440	220	(Blood glucose six hours after therapy commenced)
	4	4	2.89	1.20-6.98	10	480		
	5	8	5.16	4.30-6.21	16	520	88	(Blood glucose fifteen hours after therapy commenced)
	6	8	8.36	7.36-9.48	19	530	—	
Jon	*Control‡*	*13*	*5.15*	*4.52-5.92*				
45,	1	12	4.69	3.39-6.49	0	0	1,570	CO_2=11
Fe	2	11	9.33	7.96-6.90	3	300	910	
	3	7	7.20	5.64-9.18	4	300	—	
	4	8	6.31	4.72-8.43	5	500	—	
	5	8	8.79	6.15-12.60	6	600	472	
	6	7	4.70	2.99-7.40	16	660	420	
And	*Control*	*4*	*3.44*	*2.96-3.98*				
18,	1	10	3.47	2.41-4.99	0	0	632	
Fe	2	10	6.30	5.72-6.93	3	200	262	
	3	8	4.35	2.31-8.18	6	400	340	
	4	8	6.17	4.71-8.07	15	500	200	
	5	4	4.89	3.41-7.00	20	570	280	
Owe	*Control*	*6*	*3.76*	*2.68-5.27*				
40,	1	15	3.44	2.72-4.35	0	0	518	
Fe	2	11	5.42	4.40-6.68	3	400	90	
	3	8	5.01	3.78-6.64	6	600	35	
	4	8	7.02	5.21-9.44	8	640	210	
	5	4	3.18	1.36-7.45	16	730	114	
Sut	*Control*	*4*	*3.98*	*2.41-6.55*				
16,	1	6	4.82	1.39-6.17	0	0	456	
Ma	2	14	5.36	4.34-6.65	2	100	194	
	3	11	5.22	4.09-6.70	6	200	103	
	4	8	8.41	3.94-17.96	9	350	—	
	5	4	3.18	2.56-18.72	18	—	—	
Cro,	*Control*	*2*	*1.60*					
19	1	4	2.10	0.71-6.38	0	0	365	
Ma	2	9	2.15	1.90-2.43	2½	200	170	
	3	8	2.37	1.91-2.93	5	300	140	
	4	4	7.64	4.26-13.70	7	500	92	
Pra	*Control*	*4*	*2.73*	*0.88-8.49*				
Fe	1	10	2.36	1.60-3.49	0	0	620	
	2	5	7.85	6.71-9.15	2	200	600	

*Geometric mean. †Standard error.

‡Control category for each subject includes all control determinations performed coincident with any glucose uptake determinations of that subject sera.

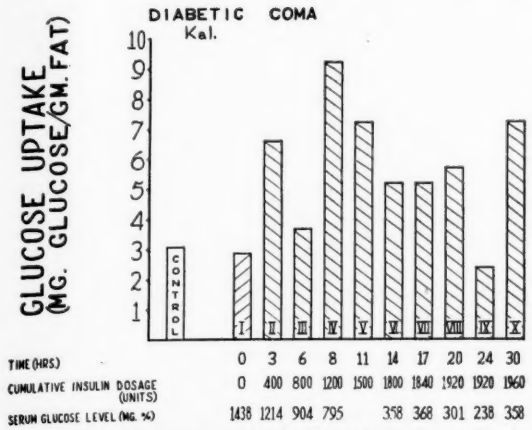

FIG. 3. Diabetic coma serum insulin-like activity of a single subject. Sera obtained before commencement of therapy and at various intervals of time following admission. Time intervals, in hours, cumulative insulin dosage, and serum glucose levels indicated. Insulin-like activity represented by antilog of geometric mean. (Control category composed of all control determinations performed coincident with glucose uptake determinations of subject's sera.)

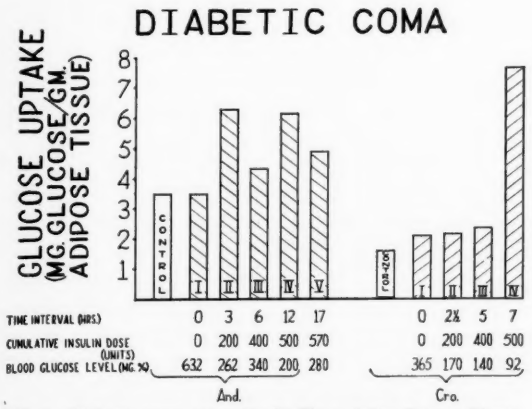

FIG. 4. Diabetic coma serum insulin-like activity of two subjects. Sera obtained before commencement of therapy and at various intervals of time following admission. Time intervals, in hours, cumulative insulin dosage, and serum glucose levels indicated. Insulin-like activity represented by antilog of geometric mean. (Control category composed of control determinations performed coincident with glucose uptake determinations of each subject's sera.)

sulin-like activity in the initial pretherapy serum specimen, the glucose uptake being 7.53 mg. glucose/gm. adipose tissue. The uptake value decreased to 2.89 mg. glucose/gm. tissue ten hours after therapy commenced. In the other coma cases, no insulin-like activity was evident in the pretherapy sera, the glucose uptakes showing no significant difference from control uptakes (table

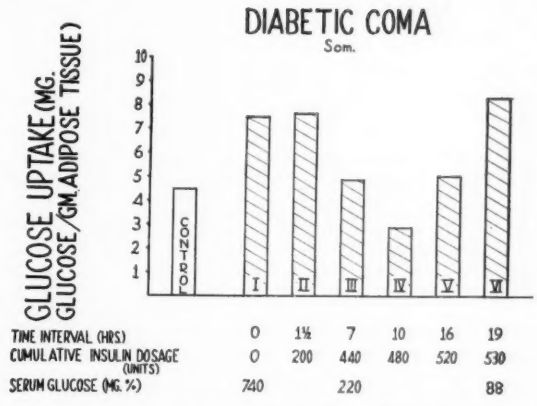

FIG. 5. Diabetic coma serum insulin-like activity of a single subject. Sera obtained before commencement of therapy and at various intervals of time following admission. Time intervals, in hours, cumulative insulin dosage, and serum glucose levels indicated. Insulin-like activity represented by antilog of geometric mean. (Control category composed of all control determinations performed coincident with glucose uptake determinations of subject's serum.)

4, figure 5). Two cases, Kal. and And., demonstrated variation in serum insulin-like activity as therapy progressed, with quite spectacular waxing and waning evident in the uptakes of Kal. (table 4, figures 3, 4). Delay of response to therapy was notable in the study of Cro., no significant increase in uptake occurring until 500 units of insulin had been administered over a seven-hour period (table 4, figure 4). The remaining four cases, Jon., Owe., Suth., and Pra., all demonstrated rapid increase of serum insulin-like activity consequent to therapy with little subsequent change during the period of high insulin dosage.

Preliminary studies suggest that a major factor influencing variations of serum insulin-like activity in some cases of diabetic coma may be the time interval between insulin administration and the obtaining of serum for insulin assay.[5] Increase in this time interval may be associated with diminished serum insulin-like activity.

DISCUSSION

The method of insulin assay utilizing glucose uptake by rat epididymal tissue has been shown to be suitable for clinical studies. It is an important advantage that the method is so highly sensitive that sera diluted one tenth demonstrate marked insulin-like activity. Serum sufficient for multiple determinations may be acquired with relative simplicity. Problems of specificity and precision, so common to bio-assay procedures, remain. Certain factors which may modify and improve the test are at present

under study.[5] However, in its present form, this method has indicated that definite insulin-like activity, of marked variability, is present in normal postprandial sera. Diabetic coma sera obtained before therapy commences usually show diminished or absent insulin-like activity, although individual specimens may demonstrate some activity. Following therapy for diabetic coma, there is, generally, marked increase of serum insulin-like activity, but marked individual variation is evident with considerable waxing and waning of serum insulin-like activity. This may be in part attributable to inherent variability of the assay procedure and to sample selection, but also probably reflects physiologic and pathologic factors.

It must again be emphasized that the term "insulin assay" is a definite misnomer, and the term "insulin-like activity" may be misleading. Objectively, the only measurement is of glucose disappearance from the incubation medium. It is inferred, probably quite correctly, that this reflects glucose consumption or uptake by the adipose tissue present in the incubation medium. The observation that crystalline insulin, in minute concentrations, accelerates or increases glucose uptake by adipose tissue should not permit the assumption that a substance as complex as human serum exerts this effect solely by possessing insulin. The presence or absence of increased glucose uptake may reflect the action within serum of insulin, of insulin-like material or of substances unrelated to insulin which may accelerate glucose uptake and of multiple serum factors which may diminish glucose uptake.[8–11] Recently, several discrete anti-insulin serum factors have been described.[12–16]

The presence of insulin-like activity in the initial specimen of one diabetic coma case, preceding onset of therapy, lends further credence to the proposition that multiple factors may exist which stimulate glucose uptake. As with other assay methods, this procedure measures a total biologic effect and multiple classes of substances may produce this effect. Also, different methods of assay for insulin-like activity definitely vary in sensitivity and probably differ in specificity of response to various substances. It is quite feasible, for example, that certain of the in vivo methods may detect insulin-like or anti-insulin factors not evident with in vitro technics, and the converse may also be true. Similarly, differences in type of tissue, animal species, buffer systems, and many less obvious variables may exert a profound effect on both specificity and sensitivity of the assay.

SUMMARY

A new method of assay for insulin-like activity is described, utilizing glucose uptake by epididymal adipose tissue from the Wistar rat. The method is sensitive to 10 micro-units of insulin/ml. Normal postprandial human sera (dil. 1/10), tested by this method, have insulin-like activity. Pretreatment diabetic coma sera did not, but post-treatment coma sera did exhibit such activity.

Considerable individual variation was observed within each category of serum. In part, this was attributable to the variability of the method, but probably also reflected physiologic and pathologic changes.

It is emphasized that this method of bio-assay, as others, measures a total biologic effect composed quite possibly of multiple insulin-like and anti-insulin factors, and conceivably of some factors totally unrelated to insulin.

SUMMARIO IN INTERLINGUA

Activitate Insulinoide De Normal E Diabetic Sero Human

Es describite un nove methodo pro le essayage de activitate insulinoide. Illo utilisa le acceptation de glucosa per histo adipose epididymal de rattos Wistar. Le methodo ha un sensibilitate usque a dece micro-unitates de insulina per ml. Normal postprandial seros human (in dilution de 1/10) manifesta un activitate insulinoide quando illos es testate per iste methodo. Seros obtenite ante le tractamento pro coma diabetic non manifestava ulle tal activitate, sed seros obtenite post ille tractamento manifestava lo.

Considerabile variationes individual esseva observate intra omne categoria de sero. Isto esseva attribuibile in parte al variabilitate del methodo sed probabilemente etiam a alterationes physiologic e pathologic.

Es sublineate que iste methodo de bio-essayage—como es le caso pro altere methodos—mesura un total effecto biologic que es possibilissimemente componite de multiple factores insulinoide e anti-insulinic e forsan etiam de factores totalmente sin relation a insulina.

ACKNOWLEDGMENT

Aided by Grant No. A-1516, National Institute of Arthritis and Metabolic Diseases, United States Public Health Service, and grants from the Los Angeles Diabetes Association and The Dazian Foundation.

Appreciation is expressed to Mrs. Rose Fischler for her technical assistance and to the House Staff of the Los Angeles County General Hospital for its cooperation.

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Recently S. Segal, J. Wyngaarden and J. Foley (*J. Clin. Invest.* 36:1383, 1395, 1957) reported a group of studies concerned with the metabolic fate, physiologic disposition, and effect of insulin on several pentoses in human subjects.

The rate constants for all the pentoses studied were similar, and ranged from 0.72 to 1.58. Actually, these constants represent the summation of the rate constants of several concurrent processes, namely renal excretion (40 to 60 per cent of the infused pentose was recovered in the urine), utilization, and intracellular penetration. When compared to similarly measured rate constants for glucose, 3.47, fructose, 3.84, and galactose, 6.93, (in the case of these hexoses renal excretion is negligible and the constant approximates that of utilization) the relatively slow rate of pentose metabolism in man becomes readily apparent.

A latent period of ten minutes elapsed between the intravenous injection of insulin and the appearance of a two- to three-fold increase in the rate of disappearance of D-xylose and L-arabinose. This accelerated rate of pentose removal lasted only twenty to thirty minutes (although hypoglycemia persisted much longer) and was followed by an abrupt return to the rate initially observed. Comparison of the blood level found thirty minutes after insulin administration with that obtained by extrapolation of the initial curve revealed differences of 30 to 35 per cent. Since urinary loss was not accelerated by insulin, these results were interpreted as indicating that the hormones accelerated the rate of pentose entry into the peripheral tissues (e.g., muscle) thereby increasing its volume of distribution in the total body water. In support of this interpretation, the authors calculated the volumes of D-xylose and L-arabinose distribution, in the presence and absence of administered insulin, and found that they were increased one and one-half times by the hormone. Under similar experimental conditions D-arabinose and D-lyxose did not respond to insulin. Because of the possibility that small changes in the rate of disappearance could occur in the post-insulin period without being detected with the single injection technic, two experiments were performed with D-lyxose and D-arabinose using a constant infusion procedure. Under these conditions D-lyxose showed a minimal response to insulin whereas D-arabinose remained unaffected. To dissociate the pentose response to insulin from the concurrent hypoglycemia, several studies were performed in which glucose was infused immediately after the injection of insulin. Neither the disappearance rate of pentoses nor their responsiveness to insulin was affected by glucose in molar ratios to pentose as high as ten. This suggests that pentoses do not compete with glucose for intracellular penetration.

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Electron Microscopy of the Rat Pancreas

Effects of Glucagon Administration

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Ingle et al.[1] reported that glucagon produced a temporary exacerbation of diabetes in partially depancreatized, force-fed rats. Cavallero and Malandra[2] found that glucagon caused only a moderate increase in the glycosuria of intact rats force-fed a high carbohydrate diet. Recently, Salter et al.[3] have produced a severe temporary diabetic state in intact, force-fed rats by repeated subcutaneous administrations of large amounts of glucagon. They reported a wide variability in the appearance of beta cells ranging from degranulation and hydropic degeneration to hypergranulation. The objective of the present investigation was to use both light and electron microscopy for a detailed study of the changes occurring in islet and acinar cells of force-fed rats treated with large amounts of glucagon. It was hoped that by utilizing the higher powers of magnification obtained by electron microscopy it would be possible to determine the ultrastructure of partially or completely degranulated beta cells, to study their mechanism of secretion and to detect early evidence of degeneration in these cells. A search for possible changes in the ultrastructure of alpha cells was also made since they are probably the site of origin of glucagon.[4] The electron microscopic studies were feasible since the identification and characterization of normal islet cells of the rat had been accomplished previously in this laboratory.[5,6]

MATERIALS AND METHODS

Nineteen intact male Wistar rats weighing 170-180 gm. were force-fed a high carbohydrate diet* (see footnote, column two) similar to that described by Reinecke et al.[7] The volume of diet administered was increased gradually over a ten-day period from 5.0 ml. twice daily to 10.0 ml. every eight hours. Ten of the rats received a total of 0.9 mg. of crystalline glucagon† (see footnote, column two) per twenty-four hours. The glucagon was suspended in corn oil in a concentration of 1.5 mg. per ml. and injected subcutaneously every six hours for seven days. The volume of diet was increased to 12.0 ml. every eight hours after three days of treatment with glucagon since the hyperglycemia and glycosuria were not severe at this time. Controls were injected with an equivalent volume of corn oil every six hours and the volume of diet was also increased to 12.0 ml. every eight hours after three days of injections. The animals were placed in individual metabolic cages with wire bottoms and were weighed daily during the treatment period. They received water ad libitum. They were killed in groups of one and two after one, two, three, five, six and seven days of injections. In one experimental and two control rats the injections and forced feedings were stopped after seven days. They were given regular food (Rockland mouse diet) for three days and then killed.

Pancreatic tissue was prepared for electron microscopy as described previously.[5,6] Tissue from the tail of the pancreas was cut into small (1 mm.) pieces and fixed in 1 per cent osmic acid-dichromate solution[8] (*p*H 7.6) for a period of one hour. They were dehydrated in a graded series of ethanol solutions and embedded in prepolymerized methacrylate at 60° C. All pancreatic tissue was processed by a uniform schedule in order to diminish the occurrence of artifacts resulting from variations in these procedures. Thick sections (2-3 μ) of pancreas were cut on a Servall microtome and islets were identified with phase microscopy. Thin sections of islets and pancreatic acini were examined in an RCA electron microscope (EMU3B). Electron micrographs were taken at original magnifications of 2,000-6,000

Presented at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco on June 21, 1958.

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*The formula in per cent for the following high carbohydrate diet was kindly supplied by Dr. J. W. Salter, Charles H. Best Institute, Toronto, Canada: Casein (Vitamin Free), 18.0; Corn Starch, 35.0; Dextrin, 17.0; Cane Sugar, 17.5; Salt Mix W, 3.5; Alphacel, 5.0; Vitamin Mix, 2.0; Corn Oil, 2.0. One hundred grams of this diet was suspended in 130 ml. of water. Ten milliliters of the fluid diet contained 6 gm. of solid.

† Crystalline glucagon lot no. 258-2348-54-2 was kindly supplied by Dr. Mary Root, Eli Lilly and Company, Indianapolis, Indiana.

diameters and enlarged photographically.

For light microscopy, portions of pancreas, liver and kidney were fixed in Bouin's and Carnoy's fluid and embedded in paraffin. The sections (4 μ) were stained with hematoxylin and eosin, chrome alum hematoxylin,[9] aldehyde fuchsin[10] and the periodic Schiff reaction for glycogen.[11] Portions of pancreas, liver and kidney were also fixed in cobalt-formol and frozen sections were stained for lipid with Oil Red O.[12]

Daily qualitative and quantitative determinations of urine sugar were made by the method of Benedict.[13] Nonfasting blood sugar determinations were done by the method of Somogyi[14] after three, five, six and seven days of treatment as well as when the rats were killed.

OBSERVATIONS

Only a moderate glycosuria was present during the first three days of treatment with glucagon (figure 1). During this interval, the rats received 10 ml. of diet every eight hours. The volume of each feeding was then increased to 12 ml. and a marked hyperglycemia with glycosuria subsequently occurred in the glucagon-treated rats, whereas the urine of the controls remained negative for glucose. The glucagon-treated rats which survived the seven-day interval lost an average of 15 gm. of body weight while the controls gained 15-20 gm. Three of the rats receiving glucagon died on the fifth day, and the remainder appeared ill and lethargic.

Pancreas one to three days: Pancreases of glucagon-treated rats were markedly congested although other organs appeared normal. By light microscopy, a moderate

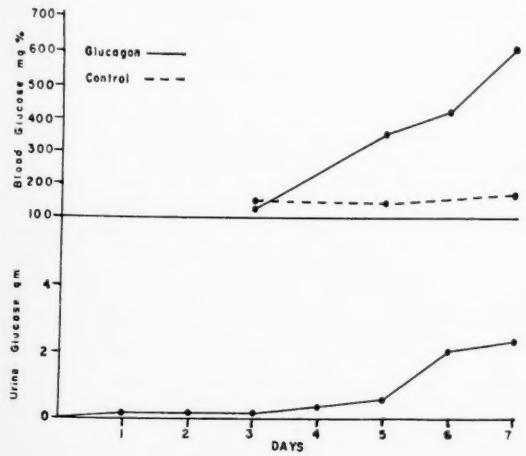


FIG. 1. Average blood and urine sugar of three glucagon-treated rats and four controls. The urine of the controls was negative for glucose during the seven-day interval.

degree of degranulation of beta cells was observed even though the hyperglycemia and glycosuria were not severe. The total amount of glucose excreted per twenty-four hours was approximately 0.6 gm. and the levels of blood sugar ranged from 162 to 230 mg. per cent in the rats killed during this interval. A comparison of the degree of degranulation and histologic changes observed in the pancreas and other organs is given in table 1. A few partially degranulated beta cells were found in control rats. Acinar cells of glucagon-treated rats were severely degranulated at one day.

The most striking alteration observed with electron microscopy was the change in size and contents of pericapillary and intercellular spaces of islets of glucagon-treated rats. In controls, these spaces were small and contained short cytoplasmic processes (figure 2) whereas in rats receiving glucagon they were markedly dilated and long cytoplasmic projections of beta cells extended into them (figure 3). In many instances, cross sections of processes were observed within the mid-portion of these spaces. Beta granules were not observed in these areas. Alpha cells, in contrast, did not develop long cytoplasmic processes and their intercellular spaces were not dilated (figure 4). The cytoplasm of some beta cells contained an abundance of a lamellar type of ergastoplasm and only a few, scattered secretory granules. In some instances these partially degranulated forms were immediately adjacent to beta cells containing a normal complement of secretory granules (figure 5). The Golgi complex and mitochondria appeared to be similar in size and distribution in these two forms of beta cells.

Pancreas five to seven days: A severe degree of degranulation of beta cells was evident by light microscopy in glucagon-treated rats (table 1). Complete degranulation had not occurred, since a few beta cells containing numerous secretory granules could be found among the degranulated forms (figure 6). Hydropic degeneration or glycogen-infiltration of beta cells was not observed in any of the sections examined. The beta cells of controls were slightly more degranulated during this interval. Acinar cells of glucagon-treated rats were again severely degranulated and decreased in size (table 1). In addition, some of these cells now contained numerous lipid droplets in the basal portion of their cytoplasm. The acinar cells containing lipid were present only in focal areas of individual lobules. Lipid was not observed in controls and the degree of granulation of their acinar cells appeared normal.

The beta cells appeared normal in size and contained an abundance of ergastoplasm (figure 7). A few, scat-

TABLE 1

Comparison of histologic changes in pancreas, liver and kidney of glucagon-treated and control rats

Group	Days	No. rats	Pancreas: Degran. β cells	Pancreas: Degran. acini	Fat	Liver fat	Kidney fat	Urine glucose gm./24 hr.*
Glucagon	1	1	3+	4+	0	0	0	0.7
	2	1	3+	3+	0	0	0	0.6
	3	1	2+	3+	0	0	0	0.6
	5	3	4+	3+	Slight	Minimal	Minimal	1.6—4.0
	6	1	3+	4+	Slight	Slight	Slight	3.0
	7	2	4+	4+	Moderate	Slight	Moderate	1.6—1.8
Control	1	1	1+	0	0	0	0	0
	3	1	1+	0	0	0	0	0
	5	2	1+	0	0	Slight	0	0
	6	1	2+	0	0	0	0	0
	7	2	1—2+	0	0	Slight	0	0
Post-glucagon	7+3†	1	1+	0	0	Slight	0	0
Control	7+3†	2	0	0	0	0	0	0

*Values on the day the rats were killed.
†Three days after glucagon injections and forced feeding had been stopped.

tered beta granules could still be found in their cytoplasm but they were apparently insufficient in number to be detected by light microscopy. The ergastoplasmic lamellae or sacs of these cells were slightly dilated. Their mitochondria and Golgi complexes did not appear altered in size or distribution (figure 8). Early evidence of vacuolation of the cytoplasm or hydropic degeneration was not found in any beta cells. A few cells containing numerous beta granules were present among the severely degranulated forms. This observation was similar to that made with light microscopy.

A distinct change in the intercellular spaces and their contents had again occurred in the glucagon-treated rats during this period. They were now small and contained only a few, short cytoplasmic projections of beta cells in contrast with the widely dilated spaces containing numerous, long processes which were observed before (figure 7 and figure 3). The alpha cells appeared to have their normal complement of secretory granules and no apparent change in their ultrastructure could be detected. The capillary endothelium of the islets was not altered in glucagon-treated rats.

By electron microscopy, a few zymogen granules could be found in the apices of acinar cells of glucagon-treated rats. Numerous, irregularly shaped lipid droplets were also present in the bases of these cells (figure 10). The size of acinar cells was reduced but their mitochondria, ergastoplasm and Golgi complexes appeared normal. Lipid droplets were not observed in the pancreas of controls and the size, degree of granulation and ultrastructure of their acinar cells remained unaltered throughout the entire period of observation.

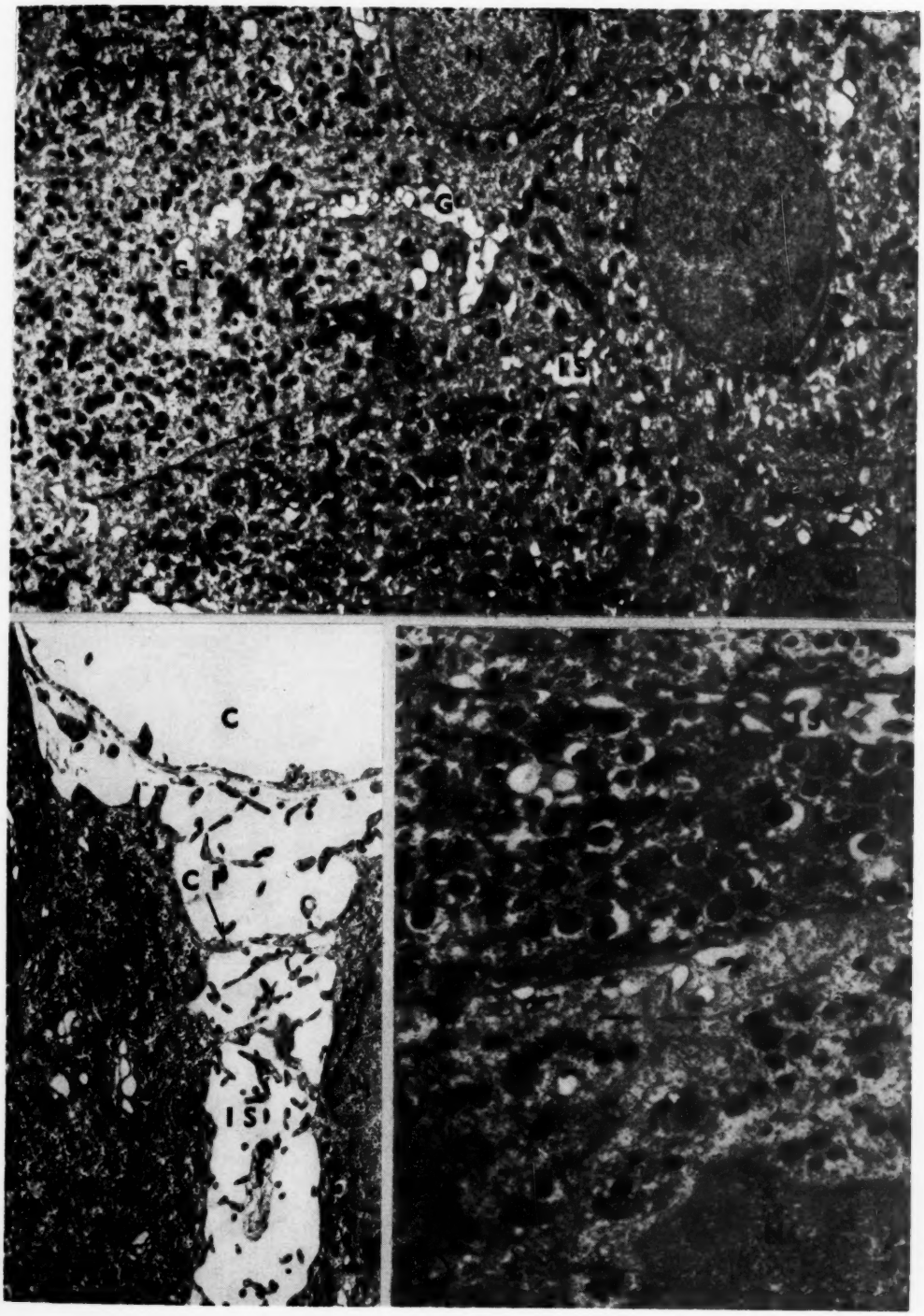
FIG. 2. The beta cells of the controls contained numerous secretory granules (GR) scattered throughout their cytoplasm. The intercellular spaces (IS) were small and contained only a few, short cytoplasmic projections of beta cells. G=Golgi complex. N=nucleus. X 8,000.

FIG. 3. The intercellular (IS) and pericapillary spaces of a rat treated with glucagon for one day are widely dilated. Long cytoplasmic processes (CP) of beta cells extend into these spaces. Cross sections of processes are present in the mid-portion of these areas. C=capillary. N=nucleus. X 8,000.

FIG. 4. Electron micrograph of portions of alpha and beta cells of a rat after two days of treatment with glucagon. The alpha cells with nuclei N_1 and N_2 are in the lower half of the illustration. The plasma membranes (arrows) of the adjacent alpha cells are closely applied and the cells do not have cytoplasmic projections. The beta cells, in contrast, have long cytoplasmic processes extending into a dilated intercellular space (IS). X 18,000.

Pancreas three days after glucagon: In one rat, glucagon injections and forced feedings were stopped at seven days. The level of blood sugar was 480 mg. per cent at this time and decreased to 170 mg. per cent three days later. The urine was negative for glucose two days after treatment was stopped. Almost complete regranulation of beta cells was evident by light microscopy. The degree of granulation of controls killed at this time was also slightly greater than in force-fed controls killed during the seven-day interval (table 1).

Numerous secretory granules were observed in beta cells with electron microscopy (figure 9). They were scattered diffusely throughout the cytoplasm. In some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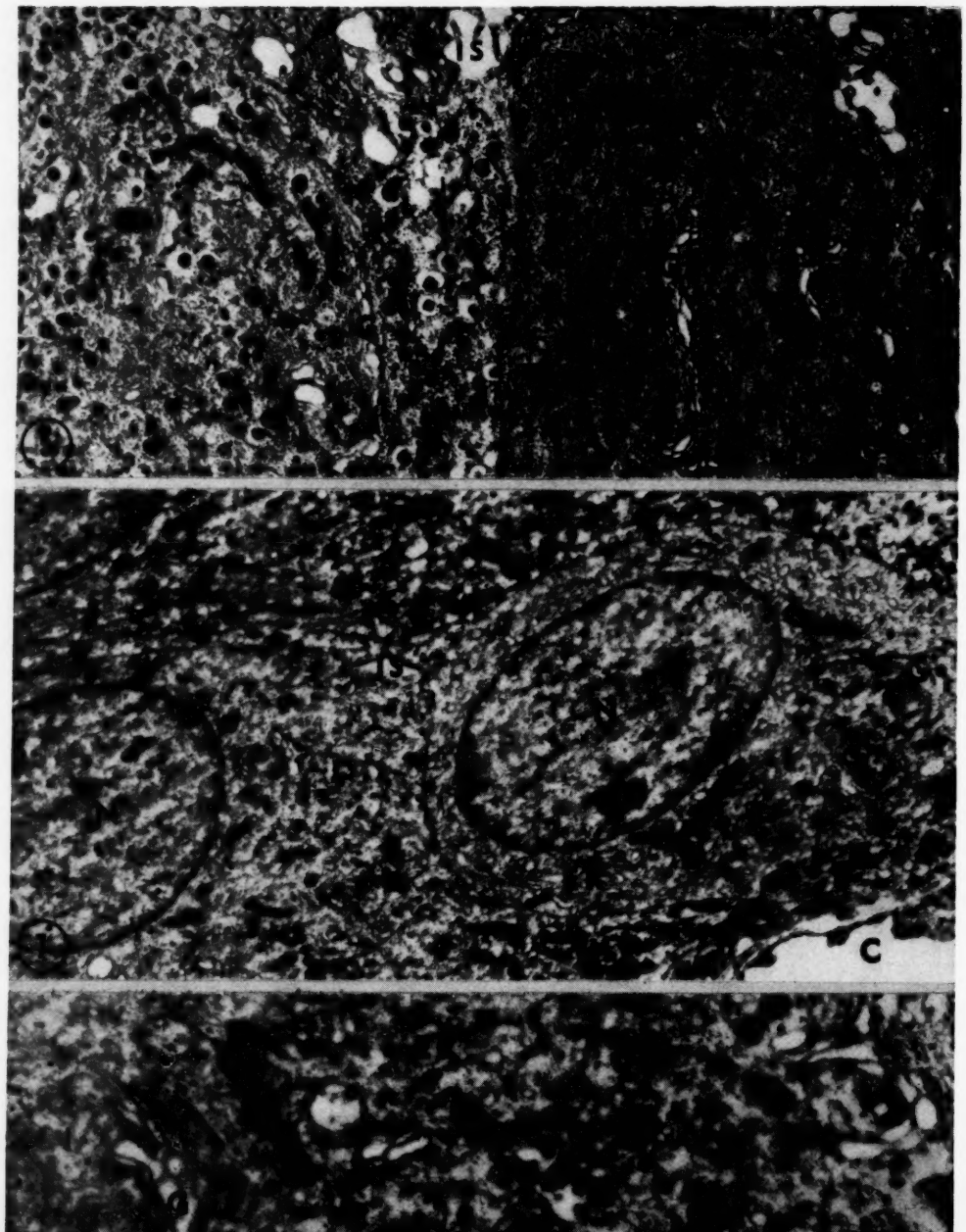
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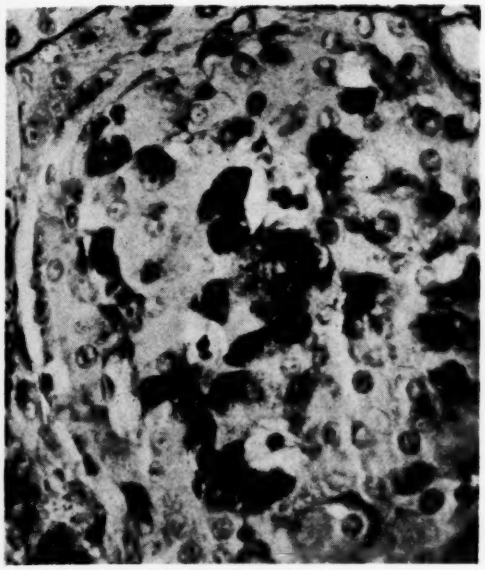

FIGURE 6

FIG. 5. Electron micrograph of portions of two beta cells from a rat treated with glucagon for two days. The beta cell on the right contains a large amount of a lamellar type of ergastoplasm (ER) and only a few secretory granules (arrow). Numerous secretory granules (arrow) are present in the other cell. The Golgi complexes and mitochondria appear similar in size and distribution in these two cells. Cytoplasmic projections of beta cells are present in a dilated intercellular space (IS). X 12,000.

FIG. 6. Photomicrograph of an islet from a rat treated with glucagon for six days. Severe degranulation of beta cells has occurred. A few cells containing secretory granules are present among the degranulated forms. Aldehyde fuchsin stain. X 540.

FIG. 7. Electron micrograph of markedly degranulated beta cells from a rat treated with glucagon for seven days. The cells contain a large amount of ergastoplasm (ER) which appears slightly dilated. A few secretory granules (arrow) were present. The mitochondria (M) and Golgi complexes (G) appear normal. The intercellular space (IS) is small and contains only a few, short, cytoplasmic projections. C=capillary. X 8,000.

FIG. 8. A portion of a severely degranulated beta cell is shown at higher magnification. One secretory granule is present and is surrounded by a distinct membranous sac. The ergastoplasmic sacs or lamellae (ER) are slightly dilated. The mitochondria (M) contain distinct cristae, and the smooth membranes of the Golgi complex are evident. These structures do not appear to be altered in size or distribution. The intact double membranes of the nucleus (N) are also shown. X 24,000.

of these cells there was suggestive evidence of a slight increase in size and distribution of the Golgi complexes. Regranulation of acinar cells and disappearance of lipid droplets were apparent by both light and electron microscopy after cessation of glucagon injections.

Liver and kidney. A slight degree of fatty metamorphosis was observed in liver cells of some of the glucagon-treated rats and in the controls during the last few days of treatment. Lipid was present in the convoluted tubules of the kidney after five days of injections with glucagon and was observed in increasing amounts during the remainder of the period of treatment (figure 11). This change was probably associated with the severe diabetic state present at this time. In the sections stained with hematoxylin and eosin, no degenerative changes could be detected either in renal tubular cells or glomeruli. Lipid was not found in the renal tubules of the rat killed three days after glucagon injections were stopped nor was it found in controls (table 1).

DISCUSSION

The results of this study confirm the findings of Salter et al.[3] that glucagon when administered in large amounts will produce a temporary diabetic state in intact force-fed rats. The characteristic changes were a marked glycosuria, hyperglycemia, body-weight loss and death of some of the rats.

Wide dilation of intercapillary spaces was one of the earliest electron microscopic changes in islets of glucagon-treated rats. Long cytoplasmic processes of beta cells projected into these spaces. The spaces did not originate as artifacts of fixation since numerous cross sections of processes could be observed within the mid-portion of these areas, which indicated that they had been present in this position prior to the time of fixation. In addition, this change was observed only in the intercellular spaces of beta cells and not in those of the alpha. During the later intervals of treatment (five to seven days), the spaces appeared normal in size and contained only a few short processes similar to those observed in normal islets. The significance of these changes is unkown but it is apparent that the processes would produce a tremendous increase in the surface area available for transfer of material into or from beta cells. Further electron microscopic studies are planned using glucose, insulin and other agents for the stimulation and inhibition of beta cell secretion in order to determine if these changes are peculiar to glucagon-induced diabetes.

Salter et al.[3] reported a variable appearance of beta cells in glucagon-treated rats ranging from degranulation and hydropic degeneration to hypergranulation. In general, progressive degranulation of beta cells was apparent with both light and electron microscopy in glucagon-treated rats of the present study. In all instances, the degree of beta cell degranulation was greater in the glucagon-treated rats than in controls, and hyper-

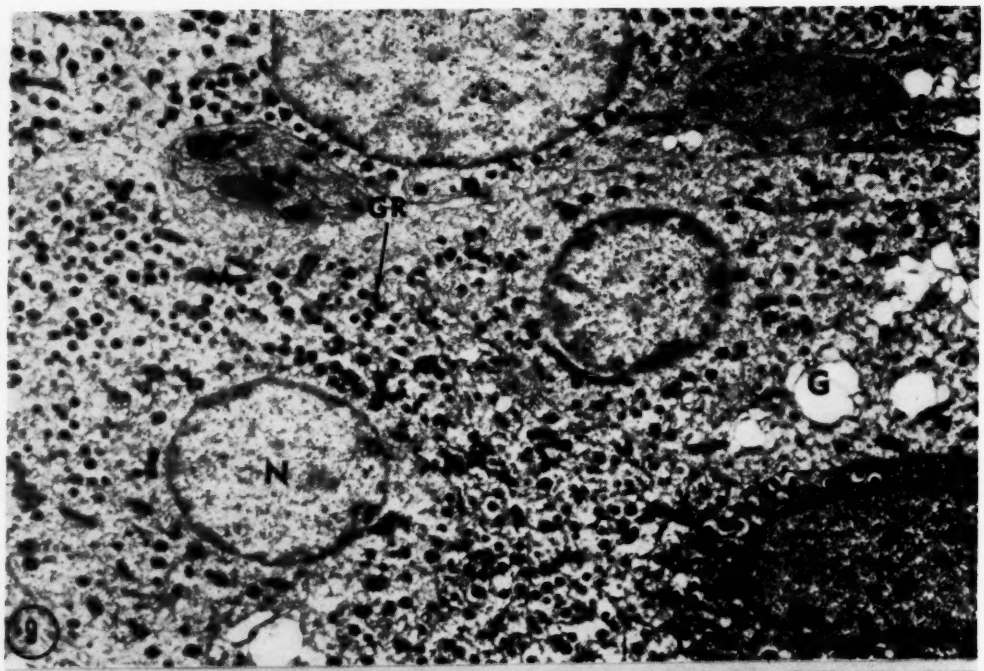

FIG. 9. Regranulation of beta cells is evident three days after stopping the glucagon injections and forced feedings. Numerous secretory granules (GR) are scattered diffusely through the cytoplasm. The Golgi complexes of these cells appear slightly dilated. N=nucleus. X 8,000.

FIG. 10. Electron micrograph of portions of acinar cells from a rat treated with glucagon for seven days. Numerous lipid droplets (L) are present in the basal portion of these cells. The cells are decreased in size and contain only a few zymogen granules (Z) in the apical portions of their cytoplasm. The slight vacuolation of the mitochondria is due to an artifact of fixation. N=nucleus. X 12,000.

granulation was not observed. Degenerative changes in the form of vacuolation, hydropic degeneration or alteration in the mitochondria were not detected with either light or electron microscopy.

In the later intervals of treatment with glucagon, nearly all beta cells appeared normal in size and contained an abundance of ergastoplasm with only a few, scattered secretory granules. Their appearance suggested an increase in the rate of formation and release of insulin at a higher equilibrium so that only a few secretory granules were being produced. These cells were apparently capable of forming secretory granules since

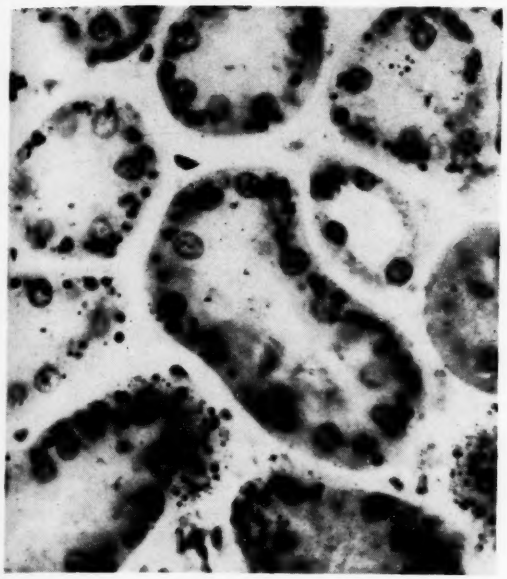

FIG. 11. Numerous lipid droplets are present in the convoluted tubules of the kidney in a rat treated with glucagon for seven days. Oil Red O Stain. X 665.

numerous beta granules were present in the islets of one rat three days after glucagon injections and forced feedings were stopped.

The mechanism by which beta cells lost their specific granules was not determined. Secretory granules or portions of them were never observed in the intercellular or pericapillary spaces. The failure to delineate the precise mechanism of secretion and regranulation was due in part to the intense stimulation of the beta cells that was obtained and the limited number of stages available for study. These mechanisms will be investigated further in the dog since the rectangular form of their beta granules[6] would provide a more distinct label for identifying granules during degranulation and regranulation.

The cytologic structure of alpha cells did not appear to be altered in glucagon-treated rats. Lazarus and Volk[15] have recently reported that a diminution in the number of identifiable alpha cells occurred in rabbits treated with glucagon and cortisone for periods up to seventeen weeks. The lack of changes in alpha cells in the present study may have been due to the relatively short period of treatment with glucagon.

Salter et al.[8] reported that degranulation and atrophy of acinar cells occurred in glucagon-treated rats. Similar findings were obtained in this study. Degranulation of acinar cells was evident as early as one day after starting treatment with glucagon. In the later intervals, focal areas of lipid accumulation were observed in acinar cells. In previous electron microscopic studies, it was found that cobalt produced similar alterations in pancreatic acini of guinea pigs.[16] The action of cobalt was believed to be toxic since it also produced degenerative changes in the mitochondria and the formation of megamitochondria in acinar cells. In the glucagon-treated rats, degenerative changes were not observed in mitochondria of acinar cells. This suggests that the action of glucagon was different from the toxic effect of cobalt. It seems unlikely that degranulation occurred as a result of the protein-catabolic action of glucagon since severe degranulation was evident early in the course of treatment. The mode of action of glucagon is obscure but the early appearance of degranulation and the lack of degenerative changes in acinar cells suggest that possibly glucagon has either a direct parasympathomimetic action or a stimulating effect on the parasympathetic nervous system. However, the pattern of acinar degranulation was different from that described by Sergeyeva[17] following vagal stimulation in cats. Peri-insular "halos" of unexhausted acinar cells were reported following vagal stimulation or choline chloride injections, whereas a uniform, severe degranulation of all acinar cells occurred in the glucagon-treated rats. Further investigations will be necessary to determine the relationship of glucagon to these interesting changes observed in acinar cells.

SUMMARY

Intact, force-fed rats receiving large amounts of glucagon were studied by light and electron microscopy. A severe hyperglycemia, glycosuria and weight loss occurred during a seven-day period of treatment with glucagon. A marked dilatation of intercellular and pericapillary spaces of islets in glucagon-treated rats was one of the earliest electron microscopic changes observed. Long cytoplasmic processes of beta cells extended into these areas. The spaces and their contents decreased in size and returned to normal during the last three days of treatment. Partial degranulation of beta cells was evident at one day and increased in severity during subsequent intervals. They contained a large amount of ergastoplasm and only a few, scattered secretory granules. Hydropic (or glycogenic) degeneration of beta cells was not observed by either light or electron microscopy. Pancreatic acinar cells were severely degranulated after only one day of treatment with glucagon and remained in this state during subsequent periods. Lipid droplets were observed in acinar cells within focal areas of pan-

creatic lobules during the last three days of injections. Lipid was also present in renal convoluted tubules during this interval. The cytologic structure of alpha cells appeared normal in glucagon-treated rats. In one rat in which forced feedings and glucagon injections were stopped, the glycosuria and hyperglycemia disappeared three days later. Regranulation of beta and acinar cells was evident at this time and lipid was not present in either acinar cells or renal tubules.

SUMMARIO IN INTERLINGUA

Microscopia Electronic Del Pancreas Del Ratto: Effectos Del Administration De Glucagon

Rattos intacte, subjicite a un dieta fortiate de grande quantitates de glucagon esseva studiate per microscopia luminar e electronic. Sever grados de hyperglycemia, glycosuria, e perdita de peso occurreva in le curso de un periodo de septe dies de tractamento con glucagon. Un dilatation marcate del spatios intercellular e pericapillar in le insulas esseva le prime observate alteration de microscopia electronic in le rattos tractate con glucagon. Longe processos cytoplasmic de cellulas beta se extendeva a in iste areas. Le spatios e lor contento decresceva in lor dimensiones e retornava a mesuras normal durante le ultime tres dies del tractamento. Disgranulation partial del cellulas beta esseva evidente al fin del prime die e cresceva in severitate durante le intervallos subsequente. Illos contineva un grande quantitate de ergastoplasma e solmente un micre numero de dispergite granulos secretori. Degeneration hydropic (o glycogenic) del cellulas beta non esseva observate per microscopia luminar e non per microscopia electronic. Pancreatic cellulas acinal esseva severmente disgranulate post solmente un die de tractamento con glucagon. Illos remaneva in iste stato durante le periodos subsequente. Guttettas de lipido esseva observate in cellulas acinal intra areas focal de lobulos pancreatic durante le ultime tres dies del injectiones. Lipido esseva etiam presente in convolute tubulos renal durante le intervallo. Le structura cytologic del cellulas alpha appareva normal in rattos tractate con glucagon. In un ratto, in que le alimentation fortiate e le injectiones de glucagon esseva arrestate, le glycosuria e le hyperglycemia dispareva tres dies plus tarde. Regranulation del cellulas beta e acinal esseva evidente a iste tempore, e lipido non esseva presente in cellulas acinal e non in tubulos renal.

ACKNOWLEDGMENT

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Embryomegaly and Increased Fetal Mortality in Pregnant Rats with Mild Alloxan Diabetes

Fredric Solomon, M.D., Chicago

INTRODUCTION

Pregnancy in diabetic women is associated with a high incidence of late intrauterine death of the fetus and perinatal mortality of the newborn. The birth weight of infants born of diabetic mothers is usually greater than that of infants born of normal mothers. The causes of the embryomegaly and high stillborn rate are not known. Attempts to reproduce experimentally clinical observations associated with diabetes in human pregnancy have had controversial and unsatisfactory results.[1-8]

Alloxan has been used for the past fifteen years to produce in experimental animals a condition analogous to human diabetes. Damage to tissues other than the β cells of the pancreas generally disappears in a few days if the animal survives.[9-12] Although transient lesions in the renal tubules have been frequently noted in alloxanized rats, Cohen[13] has shown that glycosuria and polyuria parallel the blood sugar levels in such animals.

In the experiments to be presented in this paper, diabetes of various degrees of severity was induced by the administration of alloxan to rats at various stages of gestation. When a mild form of diabetes resulted, there was a significant increase in the average fetal birth weight and an increased incidence of stillborn young. In most cases, severe diabetes resulted in early interruption of pregnancy, often preceding maternal death. Preliminary experiments in which fetuses were analyzed for moisture, protein and fat content suggest that offspring of mildly diabetic rats are large by virtue of true growth.

METHODS AND MATERIALS

A. *For effect of alloxan diabetes on pregnancy*

Fifty-three virgin albino rats of the Sprague-Dawley strain were mated with males when the females were three to four months old and weighed 204 to 233 gm. The presence of sperm on vaginal smears indicated the beginning of gestation. For the duration of the pregnancy, they were kept in individual wire-bottomed cages from which twenty-four-hour urine samples were collected for qualitative glucose determinations with Clinitest reagent tablets (copper sulfate and heating agent—Ames). The rats were allowed to eat a regular diet (Rockland Rat Diet) and drink tap water ad libitum. The animals were weighed and inspected daily.

Alloxan in 5 per cent aqueous solution was injected subcutaneously in doses of 67 to 125 mg. per kg. of body weight. In some animals, additional subcutaneous injections of 34 to 78 mg. per kg. were given after the initial single dose had failed to produce glycosuria. Eleven rats served as untreated pregnant controls.

Individual fetal weights were taken immediately after delivery, before nursing or cannibalism could take place.

B. *For comparison of composition of fetuses*

1. Some litters were used in the fetal analysis study. For purposes of convenience, analysis was carried out on four groups of fetuses in *each* litter: stillborn males, stillborn females, liveborn males, liveborn females. Groups containing more than six fetuses were subdivided. Immediately following delivery the fetus groups were weighed to the nearest 0.01 gm., wrapped in aluminum foil and placed in a freezer at —25° C. This, naturally, killed the living young; the fetuses were stored in this way for one to three months.

2. At the beginning of the analytic procedures on it, a fetus group was allowed to thaw at room temperature until moisture had ceased to condense on it and the weight of the group lay within ±0.10 gm. of the original neonatal weight. This "thawed weight" was used in later calculations.

3. Each group was then dried in a 100°-C. oven for ten to fourteen days, until the weight loss over a twelve-hour period became less than 1 mg. The percentage which moisture constituted of each group was calculated from these "dry weight" values. It was found

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Winner of the 1957-58 Medical Student-Intern Essay Contest of the American Diabetes Association for the best paper in the field of diabetes reporting original work, whether laboratory investigation or clinical observation. This is his prize-winning paper.

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that groups which were analyzed directly from the birth room, i.e., fetuses which did not go through the freezing and thawing, had percentage moisture values nearly identical with those of their frozen litter mates.

4. For the other determinations, duplicate aliquots of a homogenate of each group were used. The homogenate of a group was prepared by hydrating the dried carcasses and placing them in a high speed homogenizer (*Waring Blendor*) for two hours. The homogenization was interrupted several times to "wash down" sprayed pieces of carcass back into reach of the blades. The homogenate was finally transferred to a graduated cylinder and diluted to a convenient, recorded level—generally equal to about 100 ml. homogenate per each fetus in the group.

5. One milliliter aliquots were removed from each homogenate for determination of percentage composition of protein. Total nitrogen content in each aliquot was determined by standard micro-Kjeldahl technic. The milligrams of protein in each sample were then calculated by multiplying the milligrams of nitrogen by the factor 6.25. The percentage of protein per group was then calculated.

6. For measurement of crude fat, 10 ml. aliquots of homogenate were measured into glass weighing bottles and dried in the 100°-C. oven. The residue was then simply extracted with anhydrous ether at room temperature for thirty-six hours, resulting in recovery of "ether-extractable substance" or crude fat. Only weighing bottles with glass covers and ground glass connections were used. When compared on aliquots of the same homogenate, this method yielded virtually identical results with other methods of fat extraction. No advantage was seen, therefore, in using a 3:1 alcohol-ether mixture or a heated reflux system.

7. Mineral ash determinations were done on a few groups by heating the dry residues of 10 ml. aliquots in a 550°-C. oven for four hours. The noncombustible remainder was weighed and used in the calculation of the percentage ash per group.

8. These methods of analysis are official methods for meat analysis of the Association of Official Agricultural Chemists.[14] The technic is, in general, similar to that of Salter and Best.[15]

RESULTS

A. *Normal pregnant rats*

The pregnancies of eleven untreated control animals are characterized by the data in table 1. A total of 109 young, weighing an average of 5.75 gm., were delivered after twenty-one or twenty-two days of gestation. Four fetuses (3.7 per cent) were born dead. The weight increment of the maternal tissue (equals difference between the body weight of the mother shortly after parturition and the initial body weight on the day of conception, expressed in percentage of the initial body weight) showed much variation. The weight increment was 14.6 per cent, on the average, with a range of 6.1 per cent to 23.6 per cent. There were no abortions or maternal deaths.

The necessity of obtaining control values simultaneously with experimental data is quite marked in this field, where gestation time, stillbirth rate, and average fetal weight vary significantly between different inbred strains.[2,7,35,39]

B. *Mild alloxan diabetes in pregnant rats*

Mild alloxan diabetes was induced in seven rats on the eleventh day of gestation (Group A) and in sixteen rats on the fourteenth day of gestation (Group B). The doses of alloxan in the initial subcutaneous injection varied between 67 and 80 mg. per kg. of body weight. In eight animals one or two additional injections of 34 to 78 mg. per kg. were given on the following days.

The mild diabetes was characterized by slight polyuria and constant excretion of 0.5 to 2 per cent glucose into the urine. Four animals showed glucose-free urine a few days after delivery but the other nineteen rats became permanently diabetic. All animals remained in good condition, gained weight and lactated. The average weight increment of maternal tissue was 15.8 per cent, which does not differ significantly from the control value.

The effect of mild alloxan diabetes on fetal body weights at birth and on the incidence of late fetal death is shown in table 1. A significant increase of the average fetal birth weight was observed in both groups of rats with mild alloxan diabetes as compared with control values. The difference is shown graphically in figure 1. The stillbirth rate is increased nearly sevenfold over control values in Group B, and it is 2.8 times the control rate in Group A. The latter difference, however, is barely outside the limits of statistical significance ($p=0.06$). In these animals, as well as in the controls, stillborn fetuses showed moderate variability in body weight, but, when averaged, did not display a weight different from that of their respective Groups; they are not reported separately.

In the past, experimenters have produced oversized newborn by inducing a postmature state in prolonging gestation[16,17] or by inducing a reduction of the number of young per litter.[18] It is felt that neither situation existed in the mildly alloxan diabetic series reported

TABLE 1

The effect of mild alloxan diabetes on the pregnancy and newborn of rats

	Normal controls	Group A alloxan on 11th day	P versus controls	Group B alloxan on 14th day	P versus controls
1. Number of litters	11	7		16	
2. Number of young (av.) per litter	9.9	11.0		10.1	
3. Gestation time (days)	21.6±0.185*	21.7±0.184*	>0.05 t test	22.1±0.125*	<0.05 (=0.02) t test
4. Total number of young	109	77		162	
5. Liveborn young	105	69	>0.05 (=0.06) x^2 test	121	<0.01 x^2 test
6. Stillborn young	4	8		41	
7. Stillbirth rate (per cent)	3.7	10.4		25.3	
8. Average weight of all fetuses (gm.)	5.75±0.07*	6.10±0.19*	<0.01 t test	6.39±0.09*	<0.01 t test
9. Average weight of male young	5.85	6.24		6.54	
10. Average weight of female young	5.63	5.95		6.20	

*Standard error of the mean $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

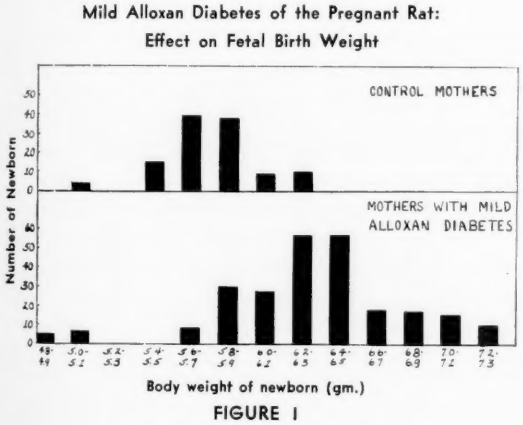

FIGURE 1

here. The number of fetuses per litter is, if anything, greater than in the control rats. On the other hand, it is true that on the average the offspring of Group B spent half a day longer in utero than offspring of controls (see line 3, table 1). However, on the basis of control values in our laboratory, it is considered "normal" for this strain of rats to deliver on either the twenty-first or twenty-second day of gestation; thirteen of sixteen Group B rats did so. The three litters which were delivered on the twenty-third day of gestation contained twenty young weighing an average of 6.27 gm., which is below the Group's average of 6.39 gm. There were three stillbirths (15 per cent). Furthermore, there is a 10 per cent probability ($x^2 = 2.71$) that three gestations of twenty-three days could have occurred by chance.

C. *Severe alloxan diabetes in pregnant rats*

Severe alloxan diabetes, characterized by excessive polyuria and pronounced glycosuria, was induced in twelve pregnant rats on the third to eighth day of gestation (Group C) and in seven animals on the eleventh to sixteenth day of gestation (Group D). The dosage of alloxan in the initial injection ranged between 70 and 125 mg. per kg. of body weight. In six animals an additional dose of 40 to 70 mg. per kg. was injected on the following day. Very little gain in maternal weight was observed, and in several animals there was a profound weight loss.

No living young were produced by the rats with severe diabetes induced by alloxan administration early in pregnancy. Eight of the twelve animals in Group C died before the expected date of delivery, five to seven days after the diabetes was induced. Prior to each maternal death, there were clinical signs of abortion, and at autopsy total or partial reabsorption of all fetuses at their placental sites was found. One rat died undelivered on the twenty-first day of gestation. Its well-developed fetuses weighed 4.9 to 5.2 gm. and showed signs of

maceration. Three Group C rats carried their pregnancies beyond the twenty-second day of gestation. Two of these rats delivered fifteen dead young weighing 4.9 to 5.6 gm. on the twenty-fourth and twenty-fifth day respectively. The third rat delivered eight dead malformed young on the twenty-seventh day after conception.

Severe alloxan diabetes induced late in pregnancy did not cause maternal death, but there was early intrauterine death and reabsorption of fetuses in four of the seven animals in Group D. The abortions occurred five days following the induction of the severe diabetes. Three rats delivered only living young on the twenty-third, twenty-fourth and twenty-fifth day after conception respectively; the average body weight at birth of the twenty fetuses was 5.36 gm. (range: 5.15 to 5.80 gm.). All of these newborn died during the first days of extrauterine life, since the severely diabetic mothers did not lactate.

D. *Analysis of fetuses from control and mildly alloxan diabetic rats*

For this preliminary experiment, two litters from normal rat mothers were compared with two litters from rats with mild alloxan diabetes. The results of analysis for percentage composition of moisture, protein and fat are summarized in table 2. The number of determinations is clearly inadequate for statistical comparison, but inspection of these early results lends the impression of there being no demonstrable significant difference between the respective compositions of normal and experimental offspring. Such data suggest that the young of mildly alloxan diabetic rat mothers are large by virtue of proportional true growth, rather than by edema or excessive fat deposition.

It will be noted that when water, fat and protein determinations are combined, less than 97 per cent of total body weight for each group is accounted for. The remainder is composed in part of mineral ash (2.0 to 2.5 per cent), which was determined in a few groups but not throughout because of inconvenience. Of course, there is also a combined error of at least 1 per cent in the whole procedure.

DISCUSSION

The main purpose of the experiments reported in this paper was to find suitable experimental conditions with which to produce oversized fetuses in alloxan diabetic rats. Hultquist[7] reported that some litters from rats depancreatized at various stages of pregnancy contained gigantic fetuses. Some large young from alloxan diabetic rats were similarly observed by Bartelheimer and Kloos.[8] Most of the gigantic fetuses in both experiments were stillborn.[7,8] H. C. Miller, on the other hand, found no abnormalities in ten pregnancies of rats with pre-existing mild alloxan diabetes.[5] Friedgood and A. A. Miller injected pregnant rats with alloxan four days prior to parturition and noted no effect.[1]

In our own experiments, mildly alloxan diabetic rats excreting 0.5 to 2 per cent glucose into the urine and remaining in good condition delivered young weighing significantly more than normal controls. The stillbirth rate in these rats is similar to the 18 per cent rate found in the alloxan diabetic rats of Barns[6] and is comparable to the 15 to 30 per cent stillbirth rate in human diabetics.[19–23] Our stillborn fetuses were not necessarily larger than their litter mates.

Several workers have implicated maternal hyperglycemia as at least partially responsible for the abnormal size and fragility of newborn of human diabetics.[20,24–28]

TABLE 2

Chemical comparison of fetuses from rat mothers with mild alloxan diabetes with fetuses from normal rat mothers

	Number of fetuses	Average fetal weight (gm.)	Moisture content (per cent)	Protein content (per cent)	Fat content (per cent)
a. Diabetic mothers, Liveborn	11 (4 groups)	5.81 (5.22—6.96)	85.8 (85.7—86.0)	10.0 (9.91—10.2)	.705 (.562—1.09)
b. Diabetic mothers, Stillborn	10 (4 groups)	6.63 (5.37—7.07)	85.9 (85.3—86.1)	9.83* (9.52—10.9)	.693 (.614—.870)
c. Diabetic mothers, live and dead combined	21 (8 groups)	6.20	85.8	9.95*	.699
d. Normal mothers, All live	23 (5 groups)	4.98 (4.66—5.42)	87.4 (87.3—87.4)	8.64 (7.96—10.1)	.668 (.493—.859)
e. Difference between c and d		+1.22	−1.6	+1.31 or +0.19 gm. per fetus	+0.021

*These values do not include a group of five dead females containing 4.02 per cent protein. The average fetal weight, moisture and fat content of this group fall well within the stated ranges for those determinations.

The many reports of hypertrophy and hyperplasia of the islets of Langerhans in stillborn young of diabetic mothers[27-29] along with the frequent clinical finding—indeed, danger—of hypoglycemia in the liveborn offspring of diabetic mothers[19,30] have suggested that these young develop with chronic intrauterine fetal hyperinsulinism. Salter and Best[15] have described the growth-promoting properties of insulin. Hultquist reported increased islet tissue in his gigantic fetuses from depancreatized rats.[7]

On the other hand, Potter et al.[31] reviewed autopsies from several centers and cast serious doubt on the significance of the finding of proliferated fetal islet tissue. Furthermore, it has been shown that both fetal and neonatal mortality[32,33] and birth weight[34,35,28] are significantly elevated in prediabetic women, when maternal hyperglycemia is not demonstrable. Given et al.[21] present evidence that rigid control of the blood sugar in pregnant diabetics does not lower the stillbirth rate and does not reduce the incidence of large babies, as compared to nonacidotic, hyperglycemic women.

Barns[6,18] and Gilbert[36] have suggested that overproduction of maternal growth hormone causes fetal gigantism in prediabetic and diabetic women. In 1935 Watts[37] injected growth hormone into pregnant rats and produced large fetuses without an accompanying reduction of the number of fetuses per litter. Barns[18] and others[38] have been unable to duplicate these results.

In addition to factors in the maternal environment, a genetic element in embryomegaly has been introduced by Jackson,[22] who reported greater than normal birth weight in offspring of diabetic fathers. Prediabetic and diabetic women have even larger babies, however, and an elevated stillbirth rate not found in the diabetic father group. In our experiments, of course, it was possible to produce large fetuses in the absence of predisposing hereditary factors from either parent.

Hoet[24] has claimed that adrenal hypercorticalism during pregnancy plays an important role in fetal nutrition. Cortisone administered in small doses to pregnant rabbits favored passage of glucose from mother to fetus —thereby "activating" fetal growth, according to Hoet's view. On the other hand, Davis and Plotz[39] found normal fetal growth, inhibited maternal growth and an elevated stillbirth rate in nondiabetic pregnant rats treated with intramuscular cortisone, 3 mg. per day. No effects were observed with lower doses. It has been found in our laboratory that there is no additional increase in the embryomegaly or high stillbirth rate of mildly alloxan diabetic rats when they are given 3 mg. of cortisone per day—i.e., cortisone has no demonstrable effect on the findings reported here in table 1.[40]

The adrenal cortex enlarges and apparently increases its function in rats made diabetic with alloxan.[41-43] Since in the experiments of Davis and Plotz[39] the administration of cortisone has consistently resulted in increased fetal mortality, it seems possible that increased adrenal cortical activity in rats made alloxan diabetic during pregnancy might account for the lethal effect on some offspring of such animals. Field[44] in acute experiments has prevented adrenal cortical hypertrophy by regulating the alloxan diabetes with insulin. It would seem that experiments are called for in which the pregnancies of adrenalectomized and alloxanized animals are observed, with and without insulin treatment.

In addition to the increased growth of the islets of Langerhans noted above, other variable characteristics of large young from human diabetics which have been reported include: edema, excessive fat deposition, hyperplasia of various endocrine organs, increased bone length, and splanchnomegaly.[20,27,29] Preliminary results of our fetal analyses tend to associate only proportional "true" growth with the embryomegaly in young of mildly alloxan diabetic rats.

It must be noted that the very great abortion rate of the *severely* diabetic animals in our experiments is consistent with the work of Davis, Fugo and Lawrence;[2] the impairment of lactation confirms the reports of Sinden and Longwell[4] and Barns, et al.[6] There is a discrepancy, however, between our results and those of others[4,5] who report successful termination of normal pregnancies in rats with permanent severe diabetes which was induced with alloxan before mating. It seems possible that the initial phase of the severe diabetic state may be more deleterious than the subsequent phase for the maintenance of pregnancy in the rat.

Alloxan is a small molecule capable of crossing the placental barrier. One might ask, then, "Are these changes in fetal growth and viability the result of some direct action of alloxan on the developing embryo?" A definitely negative answer to this question would be possible only after extensive confirmation of Hultquist's report on the offspring of depancreatized rats.[7] However, it may be noteworthy that in the course of our experiments several animals died twenty-four to forty-eight hours after receiving the initial injection of alloxan; although these rats showed gross signs of toxic damage to liver and kidney, their intrauterine contents had remained intact.

SUMMARY

Alloxan diabetes of varying degrees of severity was induced in rats at various stages of gestation. Mildly

diabetic rats delivered young with birth weights significantly larger than control values; an increased incidence of stillborn young was also observed. Severe diabetes caused early interruption of pregnancy in most cases, and no large fetuses were delivered by these rats.

Gross chemical analysis was performed on a small number of fetuses from mildly diabetic rat mothers. Virtually the same proportions of moisture, protein and fat were found in these fetuses as were found in offspring of normal rats.

Several proposed explanations of the embryomegaly and high fetal mortality associated with pregnancies of human diabetics are discussed, in light of this experimental work and that of others.

SUMMARIO IN INTERLINGUA

Embryomegalia E Augmento Del Mortalitate Fetal In Rattas Pregnante, Levemente Diabetic Per Alloxano

Diabete de varie grados de gravitate esseva inducite per medio de alloxano in rattas a varie stadios del gestation. Rattas levemente diabetic parturiva juvenes con pesos natal significativemente supra le valores de controlo. Un augmento del numero de morte-natos esseva etiam observate. Diabete sever causava le precoce interruption del pregnantia in le majoritate del casos. Le fetos parturite per iste rattas non esseva anormalmente grande.

Grossier analyses chimic esseva effectuate in un micre numero de fetos ab rattas-matre levemente diabetic. In iste fetos, quasi le mesme proportiones de humiditate, proteina, e grassia esseva trovate como in le prole de rattas normal.

Es discutite—in le lumine de iste experimentos e del experimentos de altere autores—plures del explicationes proponite pro le embryomegalia e le alte mortalitate fetal que es associate con pregnantia in diabeticas human.

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Carbohydrate Tolerance of Tube-Fed Rats

Main Effects and Interactions of Insulin, Growth Hormone, and Carbutamide

John A. Owen, Jr., M.D., with the technical assistance of James L. Bland, B.S., Augusta

The much-discussed mode of action of the hypoglycemic sulfonylureas is still not clearly understood, but accumulated evidence so far favors the theories of (1) increased pancreatic insulinogenesis, or (2) decreased hepatic glucose release, not that these need be mutually exclusive. Fritz et al. have pointed out that if these drugs produce hypoglycemia by stimulating the beta cells to secrete more insulin, their prolonged administration might eventually result in beta-cell exhaustion and impaired carbohydrate tolerance.[1] Indeed, experiments have already demonstrated this effect in rats and dogs.[2] There is also evidence that the sulfonylureas have little or no hypoglycemic effect in animals with well-established steroid or growth hormone diabetes.[3-5]

In a previous study it was possible to overcome the intact rat's well-known resistance to growth-hormone diabetes in four days by the simultaneous challenges of a high carbohydrate load and large doses (2 mg./100 gm. body weight) of growth hormone, neither of which was diabetogenic alone.[6] It seemed reasonable to think that this dual stimulation had led to temporary beta-cell exhaustion. It followed that the addition of carbutamide to this regimen might achieve a triple stimulation and result in a more severe temporary diabetes; on the other hand, the addition of insulin would be expected to give the beta cells some protection from the dual or triple stimulation. The present study was designed to test these speculations, and included quantitative measurements of glycosuria, nitrogen balance, and weight gain as corollary indices of the growth hormone effect as regards either diabetes or growth. By use of a factorial design and the analysis of variance, it was possible to evaluate statistically the main effect of each single treatment on each parameter as well as the interaction of any two or more treatments.

The results obtained fail strikingly to substantiate the speculations mentioned, but give rise in turn to other speculations, which will be discussed briefly.

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METHODS AND MATERIALS

Male albino rats of the Wistar strain were housed in individual metabolism cages in an air-conditioned room and received water ad libitum plus a liquid diet administered twice daily via stomach tube. The diet composition is shown in table 1.

TABLE 1

Composition of liquid diet

Sustagen (Mead Johnson)	2,700 gm.
Corn starch (Staley Mfg. Co.)	1,400 gm.
Dextrose, U.S.P. (Merck)	775 gm.
Alphacel (Nutritional Biochemical Corp.)	336 gm.
Salt Mixture W. (Nutritional Biochemical Corp.)	160 gm.
Tetracyn-SF (Pfizer)	9 gm.
Menadione (Nutritional Biochemical Corp.)	400 gm.
P-Aminobenzoic acid (Nutritional Biochemical Corp.)	560 mg.
i-Inositol (Nutritional Biochemical Corp.)	1,700 mg.
Water, q.s. ad	9,000 ml.

This diet is essentially that used in previous studies;[7] it supplies 0.44 gm. carbohydrate, 0.07 gm. protein, 0.01 gm. fat, and 2.13 calories per ml. Three minor changes may be noted: (1) In the previous diet the carbohydrate calories were divided as follows: starch 33 per cent, dextrin 33.5 per cent, and dextrose 33.5 per cent; the present division is: starch 35.2 per cent, dextrin 12 per cent, maltose 16 per cent, lactose 14.6 per cent, and dextrose 22.2 per cent; (2) the bulk (Alphacel) content is lower in the present diet, 3.7 per cent as compared with 5.3 per cent, with a gratifying decrease in viscosity; and (3) the inclusion of vitamins in the Sustagen and Tetracyn-SF insures supplementation equal or superior to that achieved previously. The present diet is also much simpler to prepare and easier to administer.

Each rat was adapted gradually to tube feeding and was gaining weight on a diet volume of 22 ml. B.I.D. at the start of the experiments. Animals were assigned

at random to each of eight treatment schedules (table 2). The experiment consisted of four series of eight rats each, studied over a period of two control days followed by four treatment days.

TABLE 2
Design of experimental groups

Treatment	HGF-free insulin (units/100 gm. body wt.)	Growth hormone (mg./100 gm. body wt.)	Carbutamide (mg./100 gm. body wt.)
1	—*	—*	—*
2	0.1	—	—
3	—	1.5	—
4	0.1	1.5	—
5	—	—	5.0
6	0.1	—	5.0
7	—	1.5	5.0
8	0.1	1.5	5.0

*Denotes injection of equivalent volume of normal saline.

HGF-free insulin (unmodified) was diluted to a concentration of 2 units/ml. with physiologic saline and injected subcutaneously in the left flank one hour after the morning tube feeding on treatment days 1-4. The growth hormone (NIH-BGH-1) is a highly purified bovine preparation being distributed by the National Institutes of Health. It was dissolved (10 mg./ml.) in distilled water and injected subcutaneously in the right flank immediately after the morning tube feeding on treatment days 1-4. Carbutamide was supplied in solution (50 mg./ml.) and was injected intraperitoneally one hour after the morning tube feeding on treatment days 1-4. The volume, route, and time of control injections of physiologic saline were always the same as for the appropriate drug.

Daily weights were recorded each morning followed by tube feeding and injection on treatment days 1-4. Three hours after tube feeding, 0.2 ml. of blood was obtained from the tail without anesthesia (control days 1-2 and treatment days 3-4) and analyzed in duplicate by a glucose oxidase colorimetric method. This method involved the use of the Somogyi-Nelson[8] filtrate and the commercial reagent Glucostat* with minor modifications of the directions furnished by the manufacturer. It has been previously evaluated and found to be almost completely specific for glucose.[9] A complete urine and stool collection was made daily. For each rat, forty-eight-hour pooled specimens (control days, and treatment days 1-2 and 3-4), were analyzed for nitrogen content by the micro-Kjeldahl method. Aliquots, in triplicate, of each new batch of diet were similarly analyzed. The quantitative method of Benedict[10] was used to determine the twenty-four-hour glycosuria.

*Worthington Biochemicals, Freehold, New Jersey.

Results are expressed as follows: (1) "Net change in blood sugar" refers to the mean blood sugar (in milligrams per cent) for the last two days of treatment less the mean value for the two control days; (2) "net nitrogen balance" is the mean forty-eight-hour balance (in milligrams of nitrogen) for the four treatment days less the net balance for the two control days (net balance is defined as nitrogen intake less the sum of urinary and fecal nitrogen); and (3) "net change in weight" is the mean daily gain or loss in weight (in grams) for the four days of treatment less the mean daily gain or loss for the two control days. Since the glycosuria in these studies was random and minimal, it does not appear to warrant detailed analysis.

These data are analyzed by application of the analysis of variance to a 2^3 factorial design,[11] as was done recently in a similar study that contains a detailed account of the method.[12] This method is used since it (1) is more efficient, in that the main effect of each single treatment is evaluated with the precision that would have obtained if all thirty-two animals had been used for that purpose; (2) permits the statistical appraisal of the interactions of two or more treatments as well as the main effects; and (3) has the theoretical advantage that measuring the effects of treatment under a variety of conditions supplies a broader inductive basis for the conclusions. As was true in the previous study, not all of the series were complete at the end of the study and the missing values were obtained from a later series. This nullifies any analysis of the series, without interfering with the analysis of the treatment differences.

The term "main effect" means the over-all effect of any single treatment and represents the mean difference between the sixteen animals that received that treatment and the sixteen that did not. The term "interaction" is a numerical expression of that portion of the total effect of two or more treatments which is not accounted for by the algebraic sum of their main effects; if positive, it suggests synergism; if negative, it suggests competition.

RESULTS

During the baseline studies, the mean three-hour postprandial blood glucose was 116 mg. per cent, the net nitrogen balance was +177 mg./48 hr., and the mean daily gain in weight was +1.67 gm./day. The effects of the various treatments are recorded in tables 3-5.

As regards net change in blood sugar, the calculations in table 3 show that carbutamide exerted a significant hypoglycemic effect ($P<0.01$) and that growth hormone

TABLE 3

Main effects and interactions of the treatments (table 2) as regards net change in blood glucose

Series	Treatments								Total
	1	2	3	4	5	6	7	8	
1	22	3	43	57	—24	—47	4	—7	51
2	29	—20	19	14	—7	12	17	16	80
3	—15	—17	32	26	—9	—78	23	8	—30
4	11	—11	39	25	—18	—14	5	23	60
Total	47	—45	133	122	—58	—127	49	40	161
Mean	11.75	—11.25	33.25	30.5	—14.5	—31.75	12.25	10	

Adjustment for mean	=	$(161)^2 \div 32 = 810.3$			
Treatment sum of squares	=	15,075.25 — 810.3	=	14,264.95	
Total sum of squares	=	23,411 — 810.3	=	22,600.70	
Residual sum of squares	=	22,600.70 — 14,264.95	=	8,335.75	
Mean error variance	=	$8,335.75 \div 24 = 347.32$			
Standard error	=	$\sqrt{347.32} \div 8 = \sqrt{43.42} = 6.5917$			

					t	P
Main effect insulin	=	¼(—2.50 — 42.75)	=	—11.3125	1.7162	<0.10*
Main effect G.H.	=	¼(86 — (—45.75))	=	32.9375	4.9968	<0.01
Main effect carbutamide	=	¼(—24 — 64.25)	=	—22.0625	3.3470	<0.01
Interaction ins. + G.H.	=	¼(37.75 — 2.50)	=	8.8125	1.3369	N.S.
Interaction ins. + carbutamide	=	¼(23.25 — 17.00)	=	1.5625	.2370	N.S.
Interaction G.H. + carbutamide	=	¼(22.75 — 17.50)	=	1.3125	.1990	N.S.
Interaction ins. + G.H. + carbutamide	=	¼(17.50 — 22.75)	=	—1.3125	.1990	N.S.

*If this is considered to be a one-tailed distribution, $P<0.05$.

produced a significant hyperglycemia ($P<0.01$). Surprisingly, the main effect of HGF-free insulin in this rather large dose was a mild hypoglycemia, barely significant at the 10 per cent level. On the assumption that the only possible effect of this preparation on blood glucose is hypoglycemia, the distribution becomes one-tailed and hence the insulin effect may be considered significant at the 5 per cent level. None of the interactions was significant. Carbutamide was completely effective, and insulin almost completely ineffective, in preventing the hyperglycemic effect of growth hormone.

In view of the rather mild hyperglycemic effect of growth hormone (mean elevation of 33 mg. per cent) it is not surprising that little glucose appeared in the urine. Calculations (not shown here) indicate that a significant glycosuria occurred only as a main effect of growth hormone treatment, in agreement with blood glucose results.

Table 4 shows the effects of the various treatments on net nitrogen balance. Growth hormone produced significant nitrogen retention, but neither of the other main effects and none of the interactions achieved significance. Both insulin and carbutamide seemed to enhance the action of growth hormone, yet the insulin-carbutamide combination resulted in nitrogen wastage.

Table 5 shows the effects of the various treatments on the net change in weight. The results resemble those obtained with nitrogen balance in that (1) only the main effect of growth hormone (weight gain) achieves statistical significance; (2) carbutamide slightly augmented the effect of growth hormone; and (3) the combination of carbutamide and insulin resulted in weight loss.

DISCUSSION

The growth-hormone diabetes observed was mild, despite previous findings of severe diabetes in similar rats receiving the same dose of hormone and the same volume of the original diet.[6] In another earlier study, rats receiving a larger dose of growth hormone (Horner's PR-3) but a generally smaller volume of the original diet showed changes in blood sugar, nitrogen balance, and weight gain closely resembling those reported here.[12] These comparisons imply that the present diet may be slightly less diabetogenic, but the difference is certainly not striking.

A mild diabetes should theoretically be easily susceptible to either ameliorating or exacerbating influences. Yet insulin (0.1 U./100 gm. body weight) had little over-all effect on the postprandial glucose. (The same

TABLE 4

Main effects and interactions of the treatments (table 2) as regards net nitrogen balance

Series	Treatments								Total
	1	2	3	4	5	6	7	8	
1	—153	—145	75	244	126	—57	113	70	272
2	72	211	376	359	183	—421	119	345	1,244
3	—267	107	—162	—115	—87	—128	560	828	736
4	272	—47	180	299	—120	—190	192	21	607
Total	—76	126	469	787	102	—796	984	1,264	2,860
Mean	—19	31.5	117.3	196.8	25.5	—199	246	316	

Adjustment for mean	$=$	$(2{,}860)^2 \div 32 = 255{,}612$
Treatment sum of squares	$=$	$1{,}017{,}739 - 255{,}612 = 762{,}127$
Total sum of squares	$=$	$2{,}241{,}410 - 255{,}612 = 1{,}985{,}798$
Residual sum of squares	$=$	$1{,}985{,}798 - 762{,}127 = 1{,}223{,}671$
Mean error variance	$=$	$1{,}223{,}671 \div 24 = 50{,}986$
Standard error	$=$	$\sqrt{50{,}986} \div 8 = \sqrt{6{,}373.25} = 79.8486$

				t	P
Main effect insulin	$=$	$\frac{1}{4}(345.3 - 369.8)$	$=$	—6.125	0.0767	N.S.
Main effect G.H.	$=$	$\frac{1}{4}(876.1 - (-161.0))$	$=$	259.270	3.2471	<0.01
Main effect carbutamide	$=$	$\frac{1}{4}(388.5 - 326.6)$	$=$	15.475	.1938	N.S.
Interaction ins. + G.H.	$=$	$\frac{1}{4}(519.3 - 195.8)$	$=$	80.875	1.0128	N.S.
Interaction ins. + carbutamide	$=$	$\frac{1}{4}(215.3 - 499.8)$	$=$	—71.125	.8907	N.S.
Interaction G.H. + carbutamide	$=$	$\frac{1}{4}(574.5 - 140.6)$	$=$	108.475	1.3585	N.S.
Interaction ins. + G.H. + carbutamide	$=$	$\frac{1}{4}(490.3 - 224.8)$	$=$	66.375	.8312	N.S.

dose of the same solution caused marked hypoglycemia in fasting rats.) In contrast, carbutamide had a significant hypoglycemic main effect. Further studies are in progress to help delineate this difference. Neither drug had any marked effect on nitrogen balance or weight gain.

The qualitative differences between the effects of insulin and the sulfonylureas have always afforded strong support to those who believe that the latter drugs produce hypoglycemia by some other mechanism than increased insulinogenesis. In fact, numerous human experiments indicate an indisputable action in decreasing hepatic glucose production. Yet in animal experiments, the evidence in favor of a pancreatic effect is just as strong. Dulin and Johnston,[13] for example, have demonstrated clearly that tolbutamide produces hypoglycemia in hepatectomized rats but not in alloxan-diabetic rats and have summarized other studies which champion the pancreatic site of action. Sobel et al. have reported similar findings in the hepatectomized dog.[14] Von Holt et al. have demonstrated an increase in plasma insulin-like activity of rats after tolbutamide administration.[4]

The results of this study can add little to the arguments for either side. Any interpretation therefore must be based on accumulated evidence from the work of others, and in the rat it does seem that most of the evidence favors an effect of the sulfonylureas on pancreatic insulinogenesis. In view of the increasing evidence of marked species differences, the results will be interpreted on that basis.

A striking finding of this study is that carbutamide is more effective than a fairly large dose of exogenous insulin in lowering postprandial blood glucose in rats whose carbohydrate tolerance is already impaired by growth hormone and a high carbohydrate diet, and there is a hint that the same may be true of protein metabolism. Assuming that this is a result of increased insulinogenesis, it must mean that the beta cells still contained ample stores of dischargeable insulin (no histopathological studies have been done to confirm this point), or that discharge of insulin into the portal circulation differs from absorption into the systemic. Furthermore, it suggests that the sequence of events must proceed from stimulus to insulin discharge through a mechanism or mechanisms different from those responsive to hyperglycemia and/or growth hormone excess, since no negative interaction of growth hormone and carbutamide was observed. It must be admitted that the difference may be quantitative rather than qualitative, since the relatively mild hyperglycemia per se may not have been a potent stimulus; the recent histopathological studies by Lazarus and Volk would support this.[5] Nevertheless this difference (fundamental perhaps to the success of

TABLE 5

Main effects and interactions of the treatments (table 2) as regards net change in weight

Series	Treatments								Total
	1	2	3	4	5	6	7	8	
1	—3.00	4.50	5.75	1.25	—1.25	—2.00	10.00	9.25	24.50
2	2.00	.25	11.00	6.50	7.50	1.25	8.00	5.00	41.50
3	2.00	—1.75	1.75	3.75	—.25	—2.50	6.75	9.00	18.75
4	.75	—2.00	5.50	5.25	—.25	—3.00	4.50	4.50	15.25
Total	1.75	1.00	24.00	16.75	5.75	—6.25	29.25	27.75	100.00
Mean	.44	.25	6.00	4.19	1.44	—1.56	7.31	6.94	

Adjustment for mean $= (100)^2 \div 32 = 312.5$
Treatment sum of squares $= 639.594 - 312.5 = 327.094$
Total sum of squares $= 838.125 - 312.5 = 525.625$
Residual sum of squares $= 525.625 - 327.094 = 198.531$
Mean error variance $= 198.531 \div 24 = 8.272$
Standard error $= \sqrt{8.272 \div 8} = \sqrt{1.034} = 1.0169$

				t	P
Main effect insulin	=	¼(9.82 — 15.19)	=	—1.3425	1.3201	N.S.
Main effect G.H.	=	¼(24.44 — .57)	=	5.9675	5.8683	<0.01
Main effect carbutamide	=	¼(14.13 — 10.88)	=	.8125	.7989	N.S.
Interaction ins. + G.H.	=	¼(13.01 — 12.00)	=	.2525	.2483	N.S.
Interaction ins. + carbutamide	=	¼(11.82 — 13.19)	=	—.3425	.3368	N.S.
Interaction G.H. + carbutamide	=	¼(14.94 — 10.07)	=	1.2175	1.1972	N.S.
Interaction ins. + G.H. + carbutamide	=	¼(14.63 — 10.38)	=	1.0625	1.0448	N.S.

sulfonylurea treatment of human diabetes) underlines the fact that temporary and incomplete imbalance in the physiologic hyperglycemia-insulinogenesis feedback mechanisms may occur in islets still containing a fair amount of insulin and still completely responsive to the sulfonylureas. It also indicates the extent of our ignorance of the physicochemical processes involved in the secretion of an intracellular hormone into the extracellular fluid.

SUMMARY

1. Intact adult rats tube-fed a high-carbohydrate diet received large doses of unmodified HGF-free insulin, growth hormone, carbutamide, and all possible combinations of these substances for a period of four days. The effects on blood glucose, nitrogen balance, and weight gain were compared to baseline values and analyzed for main effects and interactions utilizing the analysis of variance applied to a 2^3 factorial design.

2. Insulin produced a barely significant hypoglycemia, and carbutamide a highly significant hypoglycemia. Growth hormone produced significant hyperglycemia, nitrogen retention, and weight gain. No other main effects and no interactions achieved statistical significance. The insulin-growth hormone and especially the carbutamide-growth hormone interactions were always in the direction to suggest a synergistic effect. There was no evidence of competition between carbutamide and growth hormone.

3. It appears that under these conditions carbutamide stimulates insulin release from the beta cells via mechanisms which are not responsive to the hyperglycemia resulting from growth hormone administration.

SUMMARIO IN INTERLINGUA

Effectos Principal E Interaction De Insulina, Hormon De Crescentia, E Carbutamido Con Respecto Al Tolerantia Pro Hydrato De Carbon Del Parte De Rattos Nutrite A Tubo

1. Intacte rattos adulte, nutrite a tubo con un dieta ric in hydratos de carbon, recipeva grande doses de (1) non-modificate insulina libere de glucagon, (2) hormon de crescentia, (3) carbutamido, e omne le combinationes possibile de ille tres substantias durante un periodo de quatro dies. Le effectos super glucosa de sanguine, balancia de nitrogeno, e augmento de peso esseva comparate con valores de base e analysate con respecto a effectos principal e interactiones per medio del analyse de variantia applicate a un schema factorial de 2^3.

2. Insulina produceva hypoglycemia de grados a pena significative. Carbutamido produceva hypoglycemia de grados altemente significative. Hormon de crescentia

produceva grados significative de hyperglycemia, de retention de nitrogeno, e de augmento de peso. Nulle altere effecto e nulle interaction attingeva un grado de signification statistic. Le interaction de insulina e hormon de crescentia e specialmente le interaction de carbutamido e hormon de crescentia tendeva in un direction a suggerer le existentia de un effecto synergic. Nulle competition esseva evidente inter carbutamido e hormon de crescentia.

3. Il pare que sub iste conditiones carbutamido stimula le liberation de insulina per le cellulas beta via mechanismos que non responde al hyperglycemia resultante ab le administration de hormon de crescentia.

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Genetics and Glycolysis

In addition to its metabolic complexities, diabetes enjoys a peculiar position among genetically determined diseases. Empirically it is quite clear that there is a tremendous hereditary factor in diabetes, and that it is, in all probability, genetically determined. This having been considered, we are faced with the fact that the genetic transmission of diabetes cannot be explained completely using the knowledge available. Why the disease occurs at different times in life, its peculiar variability and its association with degenerative phenomena are at present unexplainable. The most likely possibility is that, as in the case of glycogen storage disease, there is a group of diseases which cannot be differentiated because the enzyme lesions are as yet unknown. When the metabolic abnormalities are unravelled we can expect to see some order in this chaos.

S. P. Bessman, in *Annals of Internal Medicine,* Vol. 48, page 1146, May, 1958.

Diabetogenic Effects of Growth Hormone

The Role of the Adrenals in Nitrogen Loss

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Houssay and Penhos[1] cite numerous studies in which anterior pituitary preparations produced diabetogenic effects in the absence of the adrenals. In hypophysectomized, adrenalectomized, partially depancreatized dogs, they observed large increases in blood sugar after somatotropin or prolactin administration, and small increases after corticotropin. Hydrocortisone also produced diabetic hyperglycemia in their animals.

Early studies by Gaebler and Robinson[2] support the contention of Houssay and Penhos that adrenalectomy does not abolish diabetogenic effects of growth hormone. Table 6 of the paper cited[2] includes two experiments in which crude growth hormone was given to a dog with pancreas, adrenals, thyroid, and parathyroids removed. Lipemia, hyperglycemia, and increase in glucosuria all occurred in the first of these experiments, while in the second there was some increase in blood sugar. None the less, there was no loss of nitrogen, such as growth hormone elicited[3] in depancreatized dogs with adrenals intact; in fact, some storage of nitrogen was induced in one of the two depancreatized-adrenalectomized animals, and both of them tolerated the growth hormone preparation much better than depancreatized ones. Thus the alleviating effects of adrenalectomy, so well established by Long and Lukens in cats,[4] and by Long in rats,[5] were again confirmed in dogs.

In the present study, we have investigated the nitrogen storing action and diabetogenic effect of a growth hormone preparation that is virtually free of corticotropin, and contains far less thyrotropic hormone than previous preparations. We have also attempted to reproduce the diabetogenic effects of growth hormone with corticotropin and hydrocortisone under our experimental conditions, and have carried out experiments to determine whether prolonged pretreatment of depancreatized dogs with hydrocortisone mitigates effects of growth hormone in a manner analogous to that observed by de Bodo and associates in hypophysectomized dogs.[6]

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MATERIALS AND METHODS

Normal and depancreatized adult bitches were employed. Dog 63, also used in a previous study,[7] was depancreatized Jan. 19, 1954, and is in excellent condition during the fifth postoperative year. Its daily insulin requirement was 36 units during most of this long history, occasionally dropping to 32 or even 28 units for periods of varying duration. Dog 73, depancreatized May 19, 1958, requires only half as much insulin as dog 63. The food intake of the two animals, given in footnotes under the tables, is similar.

The basal diet had the following composition, in percentages: casein, 36.2; cracker meal, 36.2; corn oil, 19.6; yeast, 4.0; and salt[8] mixture, 4.0. All animals received two drops of haliver oil daily, and 200 mg. of a mixture of vitamins and calcium phosphate, containing the following daily allowances of vitamins in milligrams: thiamine hydrochloride, 0.75; riboflavin, 1.5; nicotinic acid, 7.5; pyridoxine hydrochloride, 0.6; calcium pantothenate, 3.0.

Normal dogs were fed once daily. Depancreatized ones received half of the daily ration at 9 a.m., and the remainder at 3 p.m. In addition to the above supplement, they were fed 500 mg. of choline with the morning meal, and 7.5 gm. of pancreatin with each meal. As a rule, one third of the total daily dose of insulin consisted of protamine insulin, injected at 3 p.m., and the remainder of plain insulin, injected in equal amounts after the morning and afternoon meals.

Urine periods of all animals were terminated by catheterizing and washing the bladder, just before the morning feeding.

The corticotropin preparation, Acthar, Gel (Armour), was injected subcutaneously, and hydrocortisone acetate (Sharpe & Dohme) intramuscularly. One half of the total daily dose indicated in the tables was given at 9 a.m., and the remainder at 3 p.m. The bovine growth hormone preparation, Endocrine Study Section Lot R-50109, List No. 916, was dissolved in water and injected subcutaneously.

RESULTS

Data on nitrogen storage in five experiments on three normal bitches are presented in table 1. To calculate storage, we determine the average daily urine nitrogen output during a four- to eight-day control period, and integrate subsequent daily balances until the urine nitrogen returns to or exceeds its control value, whether this point precedes, coincides with, or follows termination of growth hormone injections. The amounts stored were as usual quite variable (table 1), but compare favorably with those reported previously from this laboratory.[7,9] In one of our studies (table 1 of reference 7), 5 mg. doses of growth hormone injected daily for six days induced storage of only 3.0 to 5.45 gm. of nitrogen. In an earlier series,[9] the amounts of nitrogen were larger than in the present one, since the period of injection was ten days instead of four. It is of interest, however, that the maximal fall in urine nitrogen in table 1 of the present study is similar to that which we have calculated from original data of the former one.[9] For the previous experiments (table 1 of reference 9) in which nitrogen storage was 11.76, 11.2, 18.78, 16.7, and 9.6 gm., the maximal reductions of urine nitrogen below the control values were respectively 36, 28, 34, 31, and 34 per cent.

When all our data are considered, the effect of growth hormone on nitrogen storage appears to vary less in intensity than in duration. Variability of duration accounts for many large differences in total storage, and suggests that the speed with which the homeostatic mechanism comes into play in our adult animals may be quite as important as the dose or potency of the growth hormone, in determining the total amount of nitrogen stored. Whatever the basis for variability may be, it is evident that one can not determine whether the present preparation is more potent than former ones with respect to induction of nitrogen storage. Our data do establish that storage was induced regularly, to at least the usual extent, and that the findings in depancreatized dogs, which we will now consider, are not due to peculiarities of the present lot of growth hormone. Discrepancies between nitrogen storage and other criteria of activity have been observed with some preparations.[10]

In the first experiment shown in table 2, a depancreatized dog received 7.5 mg. of growth hormone on two successive days. Since we were aware that the diabetogenic effect appears during the second twenty-four-hour period following an injection, the dose of insulin was increased from 36 to 48 units on the first day of growth hormone therapy, and to 72 units on the second day. Nitrogen storage occurred, but failed to continue, although the doses of insulin on the following three days were still large. This experiment is an almost

TABLE 1

Nitrogen storage experiments with bovine growth hormone in normal dogs

Date 1957	Dog no.	Growth hormone mg. x days	Nitrogen stored* gm.	Reduction of N output† per cent	Increase in weight† kg.
1-28	57	5 x 4	6.09	44.3	0.69
10-21	57	5 x 4	5.95	27.4	0.36
1-14	64	5 x 4	9.74	32.3	0.66
11-23	64	5 x 4	13.95	35.9	0.79
11-13	70	2.5 x 6	9.79	31.1	0.63

* Total amount per period.
† Maximal values per day during period.

TABLE 2

Experiments with growth hormone, with or without additional insulin, in depancreatized dog 63

	Date 1956—57	Insulin units/day	Growth hormone mg./day	Weight kg.	Urine sugar gm./day	Urine nitrogen gm./day
I.	12/26—12/31	36	0	17.02	< 2.3	12.61 ± 0.73
	1/1	48	7.5	17.27	< 2.0	10.66
	1/2	72	7.5	17.44	25.3	9.16
	1/3	60	0	17.33	40.1	12.00
	1/4	56	0	17.16	22.2	12.67
	1/5	48	0	17.21	5.8	12.30
	1/6—1/13	36	0	17.21	< 2.3	12.98 ± 0.85
II.	4/18—4/22	36	0	19.59	< 1.6	12.58 ± 0.37
	4/23	36	7.5	19.82	19.7	12.64
	4/24	36	7.5	18.91	75.2	14.51
	4/25	36	0	18.74	100.0	13.51

The daily nitrogen intake was 13.98 gm. during both experiments.
The daily ration consisted of 240 gm. of basal ration plus 50 gm. of sucrose during both experiments.
Numbers preceded by ± signs are standard deviations in this and subsequent tables.

exact repetition, with the present growth hormone preparation, of one carried out in the same animal with another purified preparation (table 4, experiment 1, of reference 7).

During the interval between the two experiments in table 2, the diet and insulin dosage were constant, and the animal gained more than 2 kg. in weight. In the second experiment, when 7.5 mg. of growth hormone was given, with the insulin dose constant, nitrogen loss occurred, and glucose output rose to 100 gm. during the second twenty-four-hour period following the second injection. The effect of the present preparation on insulin requirement in the first experiment, and on glucosuria in the second one, clearly indicate that it, like previous preparations, is extremely diabetogenic; in fact, it is dangerous to inject 7.5 mg. daily for more than two days. Evidently the extremely low content of corticotropin and thyrotropin has not diminished the diabetogenic effect. Another point of interest, which we have confirmed many times, is that failure to induce storage of nitrogen with growth hormone in depancreatized dogs, receiving a constant dose of insulin, occurs in animals which can gain weight and store nitrogen spontaneously under the prevailing conditions of food intake and insulin dosage.

Attempts to duplicate the diabetogenic effect of growth hormone with 20 units of corticotropin daily are presented in table 3. In the first experiment, some loss of weight and of nitrogen occurred, but glucosuria was negligible. To bring the animal nearer the threshold of glucosuria, the basal ration was increased, and supplemented with sucrose as indicated in the footnote, while the insulin dose was left unchanged. Under these conditions, corticotropin induced more nitrogen loss than before, as well as glucosuria up to 36 gm. per day. Although 20 units of corticotropin is a large daily dose, the resulting glucosuria did not compare with that elicited by growth hormone in the second experiment of table 2, particularly if one considers that insulin dosage was 36 units in the experiment with growth hormone as against 32 in the one with corticotropin.

Experiments in which two depancreatized dogs received 15 mg. of hydrocortisone daily are presented in table 4. Neither nitrogen loss nor glucosuria occurred in dog 63. In dog 73, some nitrogen loss took place but significant glucosuria was absent. In two normal dogs which received 15 mg. of hydrocortisone daily for six and eight days, the average increase in urine nitrogen during the period of treatment was 1.55 and 1.49 gm. respectively, while the maximal increase was 2.4 gm. in both animals. The relative susceptibility of normal and

TABLE 3

Experiments with corticotropin in depancreatized dog 63

	Date 1956	Corticotropin units/day	Weight kg.	Urine sugar gm./day	Urine nitrogen gm./day
I.	3/29—4/3	0	15.49	< 2.0	12.00±0.49
	4/4	20	15.40	< 2.0	12.76
	4/5	20	15.40	4.2	13.81
	4/6	20	15.34	4.0	13.24
	4/7	20	15.22	4.8	11.92
	4/8—4/13	0	15.70	< 1.0	10.37±0.53
II.	8/24—9/1	0	16.37	< 3.7	11.46±0.37
	9/2	20	16.08	< 1.0	13.39
	9/3	20	15.80	30.0	17.34
	9/4	20	15.06	36.2	17.22
	9/5	20	15.68	26.0	17.24
	9/6—9/9	0	15.74	15.8	12.05±0.70
	9/10—9/16	0	16.03	< 7.2	11.57±1.08

The nitrogen intake was 12.81 gm. daily during the first (I) corticotropin experiment and 13.98 during the second (II). During the first experiment, the daily ration consisted of 220 gm. of basal diet; during the second of 240 gm. of basal diet plus 50 gm. of sucrose. Insulin dosage was 32 units throughout.

depancreatized dogs to induction of nitrogen loss is being investigated further. The data in table 4 indicate quite clearly that no glucosuria comparable with that induced by growth hormone follows daily injection of 15 mg. of hydrocortisone. Results for 20 units of corticotropin daily were the same in dog 73 (table 4) as in dog 63 (table 3). When the dose of hydrocortisone administered to dog 63 was increased to 30 mg. daily (table 5), a decided increase in urine nitrogen occurred, but glucosuria was still very moderate.

For reasons indicated in the introduction, we also investigated the effect of prolonged administration of hydrocortisone upon the diabetogenicity of growth hormone. In the first experiment, 15 mg. of hydrocortisone were administered to dog 63 daily for eleven days. Data for the first nine days appear in line 2 of table 4. Not shown in the table is an experiment on the tenth day, when 5 mg. of growth hormone, Armour M10810, were injected. Urine glucose increased to 48 gm. on that day, and to 108 gm. on the day following. Since it was very evident that no reduction of diabetogenicity had occurred, we performed the more extensive experiment presented in table 6, using growth hormone supplied by the Endocrine Study Section. At the start of the experiment, the minimal daily insulin requirement of the animal was somewhat more than 28 units, as indicated by average daily excretion of 10.2 gm. of glucose (table

TABLE 4

Experiments with hydrocortisone and corticotropin in depancreatized dogs

Dog	Days	Hydrocortisone mg./day	Corticotropin units/day	Weight kg.	Urine sugar gm./day	Urine nitrogen gm./day
63	1-6	0	0	16.0	< 2.7	14.47 ± 0.80
	7-15	15	0	15.9	< 6.1	14.18 ± 0.97
73	1-6	0	0	15.4	< 1.0	10.79 ± 0.33
	7-12	15	0	15.0	6.8	12.80 ± 1.59
	13-20	0	0	15.7	< 1.0	10.60 ± 0.66
	21-26	0	20	15.5	8.3	14.97 ± 0.99
	27-32	0	0	16.1	< 1.0	10.86 ± 0.63

Daily insulin doses, food intake, and nitrogen intake were 32 units, 250 gm., and 14.56 gm. respectively for dog 63; and 16 units, 220 gm., 12.81 gm., for dog 73.

TABLE 5

Experiments with hydrocortisone in depancreatized dog 63

Date 1956	Hydrocortisone mg./day	Weight kg.	Urine sugar gm./day	Urine nitrogen gm./day
3/15—20	0	15.90	< 1.3	11.48 ± 0.57
3/21	30	15.86	< 1.0	12.60
3/22	30	15.86	< 1.0	13.68
3/23	30	15.51	4.0	13.52
3/24	30	15.56	9.1	15.60
3/25	30	15.56	12.7	13.40
3/26	30	15.56	9.5	12.91
3/27	0	15.40	17.8	14.35
3/28	0	15.34	8.6	12.78
3/29—4/3	0	15.49	< 2.0	12.00 ± 0.49

The daily insulin dosage, food intake, and nitrogen intake were 32 units, 220 gm., and 12.81 gm. respectively.

TABLE 6

Induction of polyuria without glucosuria in a prolonged experiment with hydrocortisone

Days	Insulin units/day	Hydrocortisone mg./day	Growth hormone mg./day	Urine sugar gm./day	Water intake ml./day
1-4	28	0	0	10.2	1,247
5-15	28	15	0	25.3	1,898
16	32	15	0	20.0	1,990
17-24	36	15	0	3.3	1,718
25-37	36	30	0	< 6.0	2,684*
38	36	30	7.5	17.5	3,400
39	36	30	7.5	53.8	3,900

Dog 63 was used in this experiment. The diet consisted of 240 gm. of basal ration.
* Highest value 3,450 ml.

6). This average value rose to 25.3 gm. when 15 mg. of hydrocortisone was injected daily for eleven days. The glucosuria was overcome by increasing the dose of insulin first to 32, then to 36 units; it did not return when the daily dose of hydrocortisone was increased to 30 mg. Polydipsia and polyuria, however, became extreme. On the thirty-fourth and thirty-fifth days of hydrocortisone injections, 7.5 mg. doses of growth hormone induced the usual severe and dangerous condition. During the night of the last day for which data are given in table 6, the animal vomited. On the following day, it refused half of its food, but still excreted 46.3 gm. of glucose, although the usual 36 units of insulin were given, and both growth hormone and hydrocortisone were discontinued.

A point of considerable interest in the experiment shown in table 6 was the occurrence, as the result of hydrocortisone therapy alone, of a condition which we have encountered in a number of experiments with growth hormone in depancreatized dogs. The animals become wildly excited, and, if water is available ad libitum, alternately drink and vomit, and are presently in very critical condition. This disturbance occurred during the thirteen-day period preceding administration of growth hormone. Although water intake was restricted sufficiently to prevent vomiting, the average intake was 2,684 ml. per day, and the highest value was 3,450 ml. Glucose output was negligible—less than 6 gm. per day.

DISCUSSION

Present findings support the idea that nitrogen loss caused by growth hormone in depancreatized dogs may be mediated by the adrenals. In our early experiments, adrenalectomy either abolished the loss, or permitted some nitrogen storage to occur;[2] in the present ones, nitrogen loss was readily induced with corticotropin in depancreatized dogs receiving constant amounts of food and insulin (tables 3 and 4). Since 7.5 mg. doses of the present growth hormone preparation contain only 0.45 milliunit (U.S.P.) of corticotropin, this exogenous source can not be implicated; it is, however, quite conceivable that injection of growth hormone could lead to discharge of endogenous corticotropin in depancreatized dogs that are not hypophysectomized. The very limited

glucosuria in our experiments with corticotropin and hydrocortisone presents no problem, if one accepts the well-supported view[11,12] that the effect of these hormones is one of accelerating gluconeogenesis. The amount of protein corresponding to the small increases in urine nitrogen would yield very little glucose.

The role of the adrenals in the induction of glucosuria by growth hormone in depancreatized dogs is more obscure. Adrenalectomy diminishes[2] but does not abolish this diabetogenic effect.[1,2] The amount of glucose excreted is quite out of proportion to the nitrogen loss. For example, in the experiment shown in table 2, excretion of 100 gm. of glucose was accompanied by a rise of about 1 gm. in urine nitrogen. Since no comparable glucosuria was induced by injected corticotropin or hydrocortisone, it would be illogical to ascribe this outpouring of glucose to a discharge of endogenous corticotropin brought about by injection of growth hormone. It appears more likely that the adrenals become involved as a result of the diabetogenic effect, and further complicate it. McArthur and co-workers[13,14] have presented evidence that insulin withdrawal brings about adrenal stimulation in some way. The doses of insulin required to correct the disturbance of carbohydrate metabolism that growth hormone causes in depancreatized dogs are so large that continuance of the usual maintenance dose may be analogous to withdrawal of insulin from a depancreatized dog not treated with growth hormone.

Scow[15] observed that whether the dose of insulin was 1 unit or 3 units per day, hypophysectomized depancreatized rats, tube-fed 7 gm. of food per day, responded to growth hormone equally well with respect to gains in weight and storage of nitrogen. A daily insulin dose of 0.3 unit was not enough to keep the animals alive and well. He believes that additional insulin is necessary for induction of maximal nitrogen storage in depancreatized cats,[16] or of any storage in depancreatized dogs[2,7] because growth hormone is more diabetogenic in these species than in rats. The role he ascribes to insulin is essentially one of controlling the exacerbation of diabetes. In the discussion section of a previous paper,[7] the present writer has stated his reasons for believing that counteraction of the unfavorable effect of growth hormone on carbohydrate utilization (a less inclusive term than exacerbation of diabetes) may not be the only role of insulin in this connection.

Spontaneous growth of an animal can be initiated or stopped by regulating the energy intake, if the diet is complete. Hormonal induction of nitrogen storage, on the other hand, is a process for which energy is drawn from sources other than surplus. Crude growth hormone preparations induced nitrogen storage in intact dogs despite very large calorigenic effects,[17] with food intake constant. Even a negative energy balance did not prevent this.[18] Growth hormone also diminished protein catabolism in fasting rats[19] and mice.[20] In depancreatized dogs, suitable combinations of purified growth hormone and insulin induce storage of nitrogen despite a rise in glucose output to 80 gm. per day; other combinations of dosages fail to induce nitrogen storage despite absence of glucosuria.[7] In all the above instances in which the matter has been investigated, increased oxidation of fat, and substitution of water for fat in tissue, removed any element of mystery so far as energetics are concerned. The findings may not, however, be entirely understood until more knowledge concerning the action of insulin becomes available from other types of experiments. Present suggestions concerning the role of this hormone in connection with nitrogen storage have the logical basis that protein synthesis requires energy. One can not overlook the fact that it also requires substrate. It seems possible that studies on the effect of insulin and other hormones on amino acid transfer[21] and retention[22] will provide important information in this connection.

SUMMARY

1. A highly purified bovine growth hormone preparation was tested in normal and depancreatized bitches. In normal animals, its nitrogen storing action was similar to that of other purified growth hormone preparations; in depancreatized animals, the diabetogenic effects were very severe, despite absence of noteworthy amounts of corticotropin or thyrotropic hormone.

2. Induction of nitrogen storage in the depancreatized dog with the present growth hormone preparation did not occur unless the dose of insulin was greatly increased, thus confirming our previous findings. The role of insulin in this connection is briefly discussed.

3. The nitrogen loss produced by growth hormone in depancreatized dogs, which was prevented by adrenalectomy in earlier experiments,[2] is readily reproduced with corticotropin or hydrocortisone; it may therefore be mediated by the adrenals.

4. Unfavorable effects of growth hormone on carbohydrate utilization in depancreatized dogs, which were reduced but not prevented by adrenalectomy in earlier experiments,[2] could not be reproduced with corticotropin or hydrocortisone.

5. Prolonged pretreatment of a depancreatized dog with hydrocortisone did not prevent occurrence of severe glucosuria when growth hormone was also given. It did cause wild excitement and polydipsia, without noteworthy glucosuria.

SUMMARIO IN INTERLINGUA

Effectos Diabetogene De Hormon De Crescentia—Le Rolo Del Corpores Adrenal In Le Perdita De Nitrogeno

1. Un purificatissime hormon de crescentia bovin esseva testate in normal e dispancreatisate canes-femina. In animales normal, le effecto del hormon super le immagasinage de nitrogeno esseva simile a illo de altere purificate preparatos de hormon de crescentia. In animales dispancreatisate, le effectos diabetogene esseva severissime, in despecto del absentia de quantitates notabile de corticotropina e de hormon thyrotropic.

2. In canes dispancreatisate, le presente preparato de hormon de crescentia non induceva immagasinage de nitrogeno, excepte quando le dose de insulina esseva grandemente augmentate. Isto confirmava nostre previe constatationes. Le rolo de insulina in iste connexion es brevemente discutite.

3. Le perdita de nitrogeno producite per hormon de crescentia in canes dispancreatisate—le qual esseva prevenite in previe experimentos per adrenalectomia—es facilemente reproducite per medio de corticotropina o hydrocortisona. Il es possibile, per consequente, que illo es mediate per le adrenales.

4. Adverse effectos de hormon de crescentia super le utilisation de hydrato de carbon in canes dispancreatisate—le quales esseva reducite ben que non prevenite in previe experimentos per adrenalectomia—non poteva esser reproducite per medio de corticotropina o hydrocortisona.

5. Le prolongate pretractamento de canes dispancreatisate con hydrocortisona non preveniva le occurrentia de glucosuria sever quando hormon de crescentia esseva etiam administrate. Illo causava un stato de grande excitation e polydipsia, sin grados notabile de glucosuria.

ACKNOWLEDGMENT

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Severe Diabetic Neuropathy

A Case Study with Special Reference to the Autonomic Nervous System

William Coleman Cohen, M.D., Baltimore

The manifestations of diabetic neuropathy may mimic a variety of neurologic diseases since it affects both peripheral and autonomic nervous systems.[1,2]

Recently a young diabetic patient was studied who showed unusually extensive involvement of the nervous system manifested principally by severe diarrhea, orthostatic hypotension, impairment of normal sweating function, and a loss of pain and thermal perception in the lower extremities. A number of diagnostic and therapeutic studies were carried out to determine the nature of the neurological deficits.

HISTORY

The patient, a twenty-three-year-old Negro male, was admitted for incision and drainage of a perirectal abscess and evaluation of diabetes.

The patient had diabetes of eight years' duration. His mother and maternal aunt were diabetic. He had adhered irregularly to a regimen of diet and insulin during most of this period. Several hospitalizations had been required for retinal detachment with repair in 1955, pneumonia in 1957, diabetic acidosis in 1957, and frequent hypoglycemic reactions. However, during the year preceding the present hospitalization, diabetic control was regarded as adequate.

Four years before the present admission the patient developed diarrhea which progressed to six to eight watery stools daily, mostly at night, and often immediately after meals. The stools were brown and liquid in character and not bulky or foul-smelling. The stools floated on the surface of the water and undigested foods, including meat particles, were frequently observed by the patient. In spite of the severe diarrhea, there was no significant loss of weight during this period.

Pain sensation had virtually disappeared in his lower extremities. Inability to sustain an erection was a prominent complaint. Moderate shooting pains involving the lateral aspect of the right leg occurred frequently and usually at night. For two years prior to admission, the patient sustained multiple third-degree burns of his lower extremities unassociated with pain while working as a baker's helper. In 1956 the patient was admitted to the neurology service of this hospital with a tentative diagnosis of syringomyelia. Principal physical findings were limited to impairment of sensory and motor functions in the lower extremities. A myelogram was reported as normal. He was discharged with a diagnosis of diabetic neuropathy.

Following release from the hospital the patient developed increasing generalized weakness and occasional syncope without associated vertigo. These symptoms were sufficiently severe to cause him to fall and sustain minor lacerations. He was unable to maintain steady employment. The patient attributed his difficulty to insulin reactions; however, these symptoms were unrelated to type or dose of insulin or to time of day. These episodes occurred consistently when the patient was standing or walking, and were relieved when he assumed a recumbent position. He had also noted a decrease in sweating, particularly in the hands and feet.

PHYSICAL EXAMINATION

The patient was a well-developed, well-nourished young Negro male, who appeared in no acute distress. The temperature, pulse, and respirations were normal. The blood pressure was 120/70 bilaterally in the supine position and 60/40 when taken erect. Weakness and syncope developed when the blood pressure was taken in a standing position. Numerous capillary aneurysms, scattered soft exudates, and a partially detached retina were noted in the left fundus. There were no abnormal findings related to the heart, lungs or abdomen. The rectal sphincter tone was poor and the bulbocavernosus reflex sluggish. Proctoscopic examination was normal.

Winner of the 1957-58 Medical Student-Intern Essay Contest of the American Diabetes Association for the best review article or case report. This is his prize-winning paper.

From the Department of Medicine, School of Medicine, University of Maryland, Baltimore, Maryland.

Many large and irregular pitted scars secondary to old burns were seen on both legs. Peripheral arterial pulsations were normal. There was some generalized decrease in muscle tone, more pronounced in the lower extremities. No muscle tenderness was elicited. There was no muscle atrophy. Deep tendon reflexes were obtunded bilaterally in the lower extremities. Pain and temperature perception were virtually absent and pin-prick and vibratory perception were diminished in the lower extremities. Superficial light touch sensation and position sense were intact. Gait was normal. The Romberg test was negative. The hands and feet were warmer than the remainder of the body. No pathologic reflexes were present.

LABORATORY STUDIES

Hematologic: Hemoglobin—10.3 gm. per 100 ml.; hematocrit—32 per cent; erythrocyte count—3.86 million/cm.^{3}; mean corpuscular volume—86 microns^3; mean corpuscular hemoglobin—27 μg.; mean corpuscular hemoglobin concentration—32 per cent. (One year previously the indices, hemoglobin, erythrocyte count and hematocrit were the same); leucocyte count—6,850/cm.^{3} with a normal differential. Because of the anemia various procedures including tests for circulating antibodies, red cell fragility, sickling preparation, hemoglobin electrophoresis, and semiquantitative urine urobilinogen were performed. The results were all within normal limits. Sternal marrow aspiration revealed moderate erythroid hyperplasia. Hydrochloric acid was present in the stomach after histamine stimulation. Intravenous infusion of ACTH resulted in a normal decrease in total circulating eosinophils.

Chemical studies of blood: Fasting glucose average—150 mg. per 100 ml.; blood urea nitrogen—12 to 21 mg. per 100 ml.; CO_2-combining power—15 to 24 mEq./L.; serum calcium—8.9 mg. per 100 ml.; serum phosphorus—4.6 mg. per 100 ml.; blood ascorbic acid—2.18 mg. per 100 ml. (normal 0.5-1.5 mg. per 100 ml.); urinary 17-ketosteroids—9.1 mg./24 hr. Serum chlorides, sodium and potassium were normal.

Tests of liver function including the Van den Bergh, serum albumin and globulin, blood cholesterol, bromsulphalein excretion, alkaline phosphatase and prothrombin time yielded normal values. The spinal fluid dynamics were normal, and the fluid was water clear. No cells were found. Protein concentration was 84 mg. per 100 ml.

Stool: The stools were grossly mushy to watery in nature and floated on water. They were not offensive in odor. Guiac test was negative and no ova or parasites were found.

Urine: The urine was entirely negative except for the presence of intermittent glycosuria. The phenolsulfonphthalein excretions in two hours in the supine and erect positions were 75 per cent and 47 per cent, respectively.

Radiologic: Examination of the chest, skull, pelvis, dorsal spine, colon, urinary tract, stomach and duodenum were normal. The small bowel pattern showed segmentation and puddling (figure 5).

Special investigations: The electroencephalogram was normal. The electrocardiogram showed nonspecific T wave changes. The response of the diarrhea to various medications was tested (table 1). The Schilling test[5] (table 2), I^{131} Triolein Tolerance Test[3,4] (figure 1), response of blood pressure to various medications (figure 2), electrical sweat test (figure 3), and skin temperature test (figure 4) were carried out.

CLINICAL COURSE

For one week staphylococcal furunculosis upset the diabetic control. The daily insulin dosage was increased from 40 to 80 units of Lente insulin and the blood glucose ranged between 39 and 336 mg. per 100 ml. Following correction of the infection, diabetic regulation was much smoother.

An attempt was made to correct the diarrhea with antispasmodic agents. With the administration of atropine (table 1), the stool frequency was reduced from six to eight, to two to three per day, and the stool became well formed. Stool fat (by Sudan III test) and undigested meat fibers were markedly reduced by this therapy. Other measures, including chlortetracycline[3] (250 mg./day for six days), placebos, multivitamins and pancreatin had no effect on the diarrhea. When furunculosis was present, no medication, including atropine, controlled the diarrhea. With resolution of the furunculosis the diarrhea was corrected with atropine (0.6 mg. four times per day).

Other defects in malabsorption also improved with atropine. Performance of the Schilling Test (table 2) demonstrated that during the diarrhea the urinary excretion of cobalt^{58} or ^{60}-labeled vitamin B_{12} was 0.6 per cent; with intrinsic factor added there was 3.0 per cent excretion. When the diarrhea was corrected with the use of atropine, there was 20 per cent excretion of the oral dose. The I^{131} Triolein Tolerance Test (figure 1) was carried out to determine if any defect in fat absorption was present. During the period of diarrhea poor absorption of the labeled fat was observed; but during the

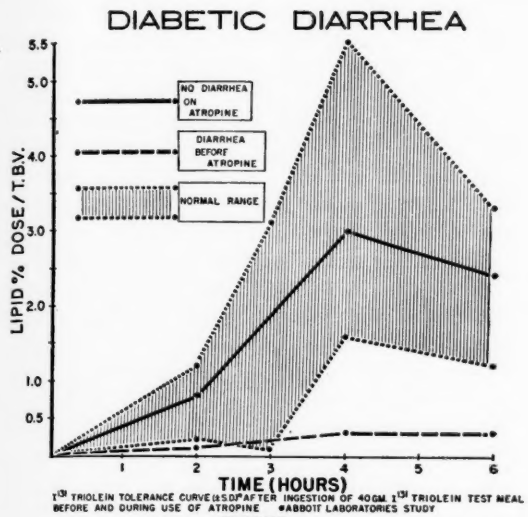

FIGURE 1

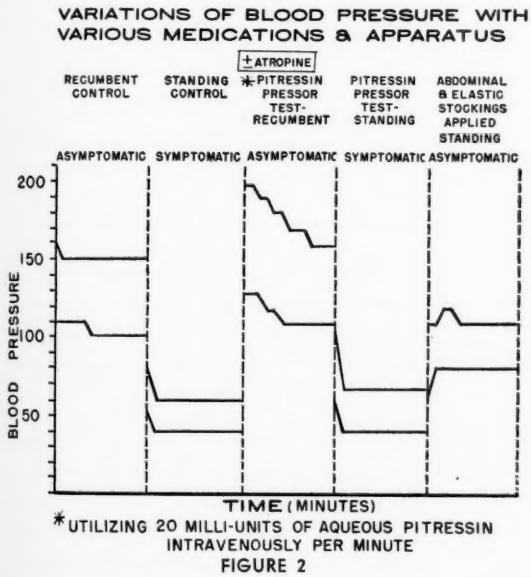

FIGURE 2

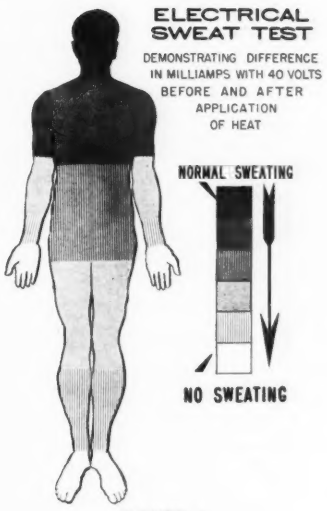

FIGURE 3

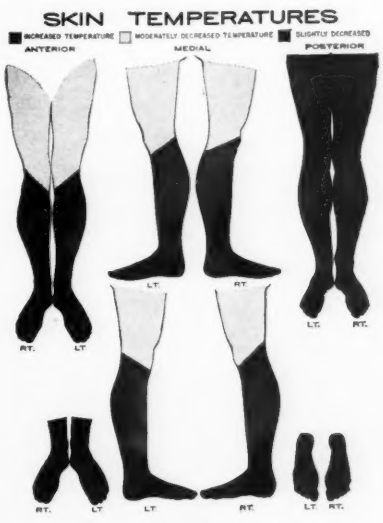

FIGURE 4

course of atropine therapy when the number of stools was reduced, a normal absorption of fat was recorded.

A number of studies of the postural hypotension were performed. When the patient assumed the erect position the blood pressure dropped from normotensive to severe hypotensive levels (figure 2). The symptoms manifested during the hypotensive state were headache, paresthesias and syncope. Unless the leg muscles were kept in constant motion while the patient was erect, he was unable to maintain the erect position without showing symptoms. Compensatory tachycardia accompanied the orthostatic hypotension. Ephedrine, atropine and intravenous pitressin[6] failed to maintain blood pressure at normotensive levels in the erect position. However, the patient remained asymptomatic in the standing position and a normal blood pressure was maintained with the use of an abdominal binder and elastic stockings. No change was noted in the peripheral neuropathy, the sweat pattern (figure 3), or the skin temperatures (figure 4) under any therapeutic regimen.

TABLE 1

Stool counts correlated with presence of undigested meat fibers and fat on various medications (six-day periods)*

	1 No therapy	2 Atropine	3 Placebo	4 Chlortetracycline	5 Pancreatin
Number of stools per day	7-8	2-3	6-8	7-8	7-8
Meat fibers	marked	normal	marked	marked	marked
Fat (Sudan III)	marked	normal	marked	marked	marked

*Note: With uncontrolled diabetes, no medications altered the stool count or decreased the amount of meat fibers and fat.

TABLE 2

Schilling Test—before and during atropine therapy

	Per cent urinary excretion	Number of stools
Cobalt^{60} Vitamin B_{12}	0.6	8
Cobalt^{60} Vitamin B_{12} and intrinsic factor (30 mg.)	3.0	8
Cobalt^{58} Vitamin B_{12} and intrinsic factor	20.0	2

Urinary excretion of cobalt^{58} (or cobalt^{60}) vitamin B_{12} utilizing a 1.0 microcurie capsule orally.

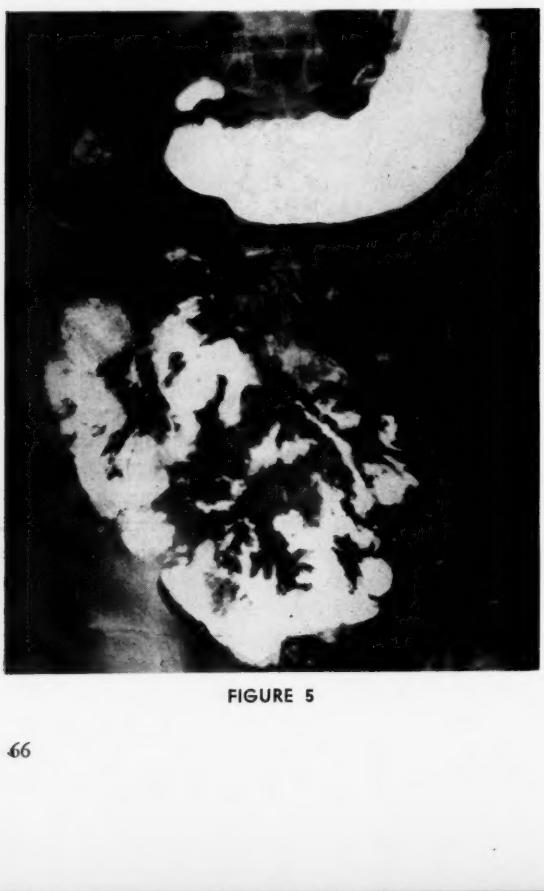

FIGURE 5

DISCUSSION

The patient exhibited an unusually severe form of diabetic neuropathy. Autonomic involvement was especially prominent as manifested by impotency, orthostatic hypotension, diarrhea and abnormalities of sweat pattern and skin temperatures. The elevation of the spinal fluid protein in this disorder has been reported previously.[7]

Frequent watery stools and nocturnal incontinence without associated weight loss were striking symptoms in this patient. The presence of excess stool fat and meat fibers is of interest and suggests inadequate pancreatic enzyme function. However the I^{131} triolein study after atropine would seem to exclude any defect in neutral fat digestion and absorption.[3,4] Malins and French[9] reported that chlortetracycline was of value in controlling diabetic diarrhea. Atropine, banthine and probanthine had no appreciable effect in their study. Chlortetracycline had no effect on the diarrhea in this patient. However, the diarrhea was controlled with atropine (table 1). During the period of reduction in number of stools per day I^{131} triolein and vitamin B_{12} absorption returned to normal.

The curve of the I^{131} Triolein Tolerance Test during the diarrhea resembles that seen in chronic pancreatitis[3,4] (figure 1). Berge, Wollaeger and associates[8] failed to find any evidence of pancreatic enzyme deficiency in diabetic diarrhea. A similar observation was noted in this patient while he was taking atropine.

The mechanism of the diarrhea in patients suffering from diabetic diarrhea has never been clearly defined. Berge, Sprague and Bennett[10] failed to find any significant pathologic change in the bowel in post-mortem examinations of patients with this disorder. Malins and French[9] stated that the fecal fat levels did not correlate well with stool volume. These authors suggested that the diarrhea was due to rapid passage of intestinal contents.

The findings in this patient support this concept, since consistent improvement was observed when the number of stools was reduced to two or three daily. In this case adequate control of the diabetes was necessary before the diarrhea would respond to medication.

In chronic diarrheal conditions progressive weight loss commonly occurs. However, in spite of severe diarrhea in this patient, weight loss did not occur. No evidence of vitamin deficiency was noted even though diarrhea had been present four years. In most malabsorption syndromes vitamin A, D and K deficiencies are common.[11]

Odel, Roth and Keating[2] noted in the diabetic with autonomic nervous system involvement, the similarity of blood pressure, skin temperature and sweating pattern to that of the sympathectomized patient. The patient herein reported demonstrated identical findings.

An abdominal binder and elastic stockings[2] were able to maintain normotensive blood pressures and to alleviate the symptoms of orthostatic hypotension. The difference observed in the phenolsulfonphthalein excretion test in the supine and erect positions was probably due to a decrease in the renal blood flow secondary to the orthostatic hypotension. The anemia was a normochromic normocytic type. The nature of the anemia remains obscure.

SUMMARY

The clinical details of a young male diabetic exhibiting severe neuropathy with involvement of the autonomic and peripheral nervous systems are presented.

Diarrhea, postural hypotension, loss of pain and thermal perception in the lower extremities, and abnormalities of skin temperature and sweat production were the dominant clinical manifestations.

The diarrhea was controlled and improvement in fat absorption demonstrated during atropine therapy. An abdominal binder and elastic stockings resulted in correction of the postural hypotension and marked symptomatic relief of the weakness and syncope. No improvement was noted in the peripheral neuropathy or in the abnormalities of skin temperature and sweat production.

SUMMARIO IN INTERLINGUA

Sever Neuropathia Diabetic: Studio De Un Caso Con Referentias Special Al Systema Nervose Autonome

Es presentate detalios clinic del caso de un juvene masculo diabetic qui manifestava sever grados de neuropathia afficiente le systema nervose autonome e peripheric.

Diarrhea, hypotension postural, perdita de dolor e de perception thermal in le extremitates inferior, e anormalitates del temperatura cutanee e del production de sudor esseva le dominante manifestationes clinic.

Le diarrhea esseva subjugate e melioration del absorption de grassia esseva demonstrate in therapia a atropina. Le uso de un bandage abdominal e de calcettas elastic resultava in correctiones del hypotension postural e in marcate grados de alleviamento symptomatic del debilitate e del syncopes. Nulle melioration esseva notate in le anormalitates del temperatura cutanee e del production de sudor.

ACKNOWLEDGMENT

I am indebted to Dr. Theodore E. Woodward and Dr. Thomas Connor for their helpful criticism, to Dr. Joseph Workman for the isotope studies, and to Mrs. M. Waldie for her secretarial assistance.

ADDENDUM

Since the completion of this paper a nine-month follow-up study revealed the peripheral neuropathy to have regressed so that pain and temperature perception are slightly diminished. Diabetic control is regarded as adequate. Atropine is still required for the control of the diarrhea, and the abdominal binder and elastic stockings are still required for orthostatic hypotension. Anemia is no longer present.

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EDITORIAL

INTERRELATIONSHIP OF GLUCOSE AND LIPID METABOLISM

Since 1948 evidence has accumulated which indicates that the synthesis of fatty acid requires the concomitant utilization of glucose. Within the past two years additional information about the relationship between glucose metabolism and fatty acid synthesis has given a better understanding of the defect in lipid metabolism in the diabetic animal. It has long been recognized that the oxidation of fatty acids is accelerated in the uncontrolled diabetic. This resulted in the investigative emphasis on ketone formation as the major defect in lipid metabolism of the diabetic patient. It was not until the work of Drury[1] and of Stetten and Boxer[2] that a marked depression in fatty acid synthesis was demonstrated. Thus, when normal glucose utilization is restricted, as in diabetes, the synthesis of fatty acids is depressed while oxidation continues unchecked.

The classic work of Lynen[3] has elucidated the steps by which long-chain fatty acids are oxidized in a stepwise fashion to form acetyl Coenzyme-A (acetyl Co-A). This so-called fatty acid cycle includes two oxido-reductive reactions which require flavin adenine dinucleotide (FAD) and diphosphopyridine nucleotide (DPN) as cofactors. It was believed that the synthesis of fatty acids resulted from the reversal of the steps of oxidation and that these same two cofactors were involved. Shaw, Dituri and Gurin[4] demonstrated that the failure of cell-free systems prepared from the livers of alloxan diabetic rats to synthesize fatty acids was due to an inability to convert acetyl Co-A to butyryl Co-A. Their work localized a possible block in fatty acid synthesis in the diabetic to the reaction in which crotonyl Coenzyme-A is converted to butyryl Coenzyme-A. Langdon[5] demonstrated that in the synthesis of fatty acids from acetate, the reduction of crotonyl Coenzyme-A to form butyryl Coenzyme-A requires reduced triphosphopyridine nucleotide (TPNH). Thus, when this reaction proceeds in the direction of the synthesis of fatty acids it requires TPNH but when this same reaction proceeds in the oxidative direction it requires FAD. This has led to the conclusion that the pathways of fatty acid synthesis and oxidation may be different.

The demonstration that fatty acid synthesis requires TPNH has aroused interest in TPNH production and its relationship to lipogenesis. Two pathways for glucose utilization exist in liver and adipose tissue, major sites of fatty acid synthesis. One pathway is the Embden-Meyerhof pathway which produces DPNH but no TPNH. The other is the phosphogluconate oxidative pathway (PGO) in which the first two reactions produce TPNH. The following evidence has led to the conclusion that the utilization of glucose via the PGO pathway is important in fatty acid synthesis. Felts et al.[6] first noted a decreased glucose utilization via the PGO pathway in liver slices prepared from alloxan diabetic rats. Siperstein[7] demonstrated in liver homogenates that the stimulation of glucose metabolism via the Embden-Meyerhof pathway by the addition of DPN to the medium results in little or no increase in fatty acid synthesis. However, when glucose utilization via the PGO pathway is stimulated by the addition of TPN, there is a marked increase in fatty acid synthesis. He also showed that the defect in fatty acid synthesis in homogenates prepared from the livers of alloxan diabetic rats can be corrected by the stimulation of glucose utilization via the PGO pathway.

Milstein[8] has also observed a decrease in the utilization of glucose via the PGO pathway as estimated from carbon dioxide production by the epididymal fat pad obtained from the alloxan diabetic rat. Winegrad and Renold[9,10] studied the relationship between glucose metabolism and fatty acid synthesis in the epididymal fat pad of the normal rat. The addition of insulin in vitro results in an increased synthesis of fatty acids from glucose at the same time that there is an increased utilization of glucose via both the Embden-Meyerhof and PGO pathways. They also showed that the stimulation of fatty acid synthesis from other precursors of acetyl Co-A (acetate and pyruvate) which results from the addition of insulin in vitro, is dependent upon the concomitant utilization of glucose. Insulin added in vitro to the fat pads of alloxan diabetic rats will correct the defect in fatty acid synthesis. Winegrad, Shaw and Renold[11] employed the in vitro effect of growth hormone in the epididymal fat pad to elucidate the relationship between a specific pathway of glucose utilization and fatty acid synthesis. When growth hormone is added in vitro to this tissue, there is an increased oxidation of glucose to carbon dioxide but no increase in fatty acid synthesis. Studies with differentially labeled glucose (glucose-1-C^{14} and glucose-6-C^{14}) indicate that growth hormone produces a marked decrease in the utilization of glucose via the PGO pathway. The increase in the carbon dioxide formation from glu-

cose makes it apparent that there is adequate formation of acetyl Co-A under these circumstances; thus a deficiency of acetyl Co-A cannot be used to explain the depressed fatty acid synthesis.

Thus, the present evidence suggests that fatty acid synthesis is dependent upon normal glucose metabolism for the production of three substances: (a) acetyl Co-A, the structural unit from which fatty acid synthesis proceeds; (b) reduced diphosphopyridine nucleotide (DPNH) which is required for most of the reductive steps in the synthesis of fatty acids; and (c) TPNH which is necessary for the reduction of crotonyl Co-A to butyryl Co-A. In diabetes, the abundant production of acetoacetic and β-hydroxybutyric acids indicates no deficiency of acetyl Co-A formation or the production of DPNH. The defect in fatty acid synthesis in the diabetic can best be explained in the following manner. Because of the insulin deficiency, glucose available for metabolism within the cell is greatly reduced and the fraction of glucose utilized via the PGO pathway is particularly depressed. Under these circumstances fatty acid synthesis from acetyl Co-A is limited by the availability of TPNH formed in the PGO pathway. It would appear that the synthesis of fatty acid is regulated by glucose utilization via the PGO pathway.

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BOOK REVIEWS

THE CLINICAL APPLICATION OF HORMONE ASSAY. *By John A. Loraine, M.B., Ph.D., M.R.C.P.* (Ed.). *$7.00, pp. 368, The Williams & Wilkins Co., Baltimore, 1958.*

This is an exceptionally valuable book. It is indeed a major undertaking to review and winnow the extensive literature pertaining to hormone assays. However, the author brings to the subject unique qualifications—a broad personal experience in hormone research, sound critical judgment and a most pleasing clarity of presentation.

In the initial chapter the general principles of hormone assay are discussed—criteria of reliability, practicability, factors entering into bio-assays and calculation of errors in bio-assays. Subsequent chapters are devoted to the individual hormones. In each chapter the several bio-assay and/or chemical assay procedures that have been published are outlined and discussed critically. In addition, the author reviews the results of assays made under normal and abnormal circumstances, a feature of particular interest to the clinician. The greatest amount of space is devoted to the gonadotropins, estrogens and progesterone, reflecting, no doubt, the author's particular interests. The discussions of the other hormones are less extensive; however, with the single exception of the chapter on insulin, which is brief (only seven pages) and less complete than one might wish, these other chapters are highly satisfactory.

In summary, this book is a most welcome addition to the book shelf, not only of the investigator interested in hormone research, but of the clinician as well.

THE NURSE AND THE DIABETIC. *By Joan B. Walker, M.D., M.R.C.S., L.R.C.P. $1.47 net (by post $1.57), pp. 128, fourteen photographs and a number of line drawings, Iliffe & Sons Ltd., London, 1958.*

This is a very well organized discussion of the management of the diabetic, with particular emphasis on the role played by the nurse. It seems quite appropriate that the discussion includes an explanation of the nature of diabetes, a description of the more routine laboratory tests, and a review of the diets and their importance. As nurses are now getting better technical training and are becoming responsible for simple laboratory tests, it is indeed fitting that this should have been discussed.

The management of the outpatient diabetic clinic is excellent and brings out the importance of having the patients seen by the staffs of other departments, such as the Eye Department, the Dental Department and the Chiropodist. A chapter also covers the diabetic emergencies that bring the patient into the hospital, and a discussion of the diabetic in the home. There is ample information on directions for taking insulin and a short discussion of the new sulfonylurea hypoglycemic drugs. This book can be recommended to nurses and to diabetic patients.

ABSTRACTS

Athanail, George; and Cabaud, Philip G. (Dept. of Pathology, The Brooklyn Hosp., Brooklyn, N. Y.): SIMPLIFIED COLORIMETRIC METHOD FOR TRUE BLOOD GLUCOSE. J. Lab. & Clin. Med. 51:321, February 1958.

The condensation of glucose with 2-aminobiphenyl results in the formation of a characteristic color, the intensity of which is directly proportional to the quantity of glucose. This color reaction may be produced with either Folin-Wu or Somogyi-Nelson deproteinized filtrates, and apparently reflects true blood glucose, although there is no evidence presented that this is so. Although statistical data are not presented, the results obtained comparing the newer technic with the standard methods seem reasonably consistent. G.J.H.

Bassøe, Hans H.; and Krogh, Hans-Kr. (Med. Dept. B and the Endocrin. Res. Lab., University Clinic, Bergen, Norway): INSULIN TOLERANCE TEST IN OVARIECTOMIZED RATS. Acta endocrinol. 28:366-68, July 1958.

Young female rats showed no significant alteration in insulin tolerance test as a result of bilateral ovariectomy. S.B.B.

Beaven, D. W. (Christchurch Hosp., New Zealand): DIABETIC COMA IN INFANCY. Brit. M. J. 2:198-201, July 26, 1958.

The author reports two cases of diabetic coma in infants under one year of age. The comparative rarity of reported cases of this age group is noted, and it is suggested that cases may masquerade as meningitis or pneumonia. The history of familial incidence may be greater than 40 per cent when accurate information can be obtained. Some aspects of the management of diabetic coma in small babies are discussed. The importance of parental intelligence and cooperation in subsequent management is stressed. C.A.R.

Birkinshaw, V. J.; Gurd, M. R.; Randall, S. S.; Curry, A. S.; Price, D. E.; and Wright, P. H. (Boots Pure Drug Co., Nottingham; Forensic Science Lab., Harrogate, Yorks; and Guy's Hosp. Med. Sch., London, England): INVESTIGATIONS IN A CASE OF MURDER BY INSULIN POISONING. Brit. M. J. 2:463, Aug. 23, 1958.

The authors report in detail the case of a young woman who was murdered by an insulin injection. So far as the authors are aware, this is the first occasion that such a charge has been substantiated and also the first in which insulin has been demonstrated in human tissue, other than the pancreas, after death. An account is given of the case in which a woman was found drowned in her bath. The post-mortem examination and the findings at the scene where the body was found suggested that prior to her death the woman was unconscious. The absence of common poisons in the tissues of the body and in the urine, the presence of vomited food on the bedclothes and in the bath, the sweat-soaked pajamas, and the grossly dilated pupils suggested that the woman was hypoglycemic. The subsequent finding of injection marks on her buttocks led to a search for insulin in the underlying tissues. A large amount of insulin was recovered (84 units), and this was thought to represent about a third of the amount present at the time of her death and an unknown lesser fraction of the amount which was injected. The woman's husband, a trained male nurse, was accused and convicted of his wife's murder. C.A.R.

Bloom, Walter Lyon (Director of Med. Education, Piedmont Hosp., Atlanta, Ga.): THE DETERMINATION OF KETONE BODIES IN BIOLOGIC FLUIDS. J. Lab. & Clin. Med. 51:824-28, May 1958.

This paper presents a modification of the Behre method for the determination of acetone, acetoacetic acid and β-hydroxybutyric acid in biologic fluids. The determination is carried out in a Thunberg tube in which the sample is placed in the tubular end along with the dichromate and the alkaline salicylaldehyde placed in the turnable side sac. The tubular end is immersed in a boiling-water bath and the side sac chilled by cold running water. Thus the acetone is distilled from the heated to the cooled end and trapped in the alkaline salicylaldehyde. Within the range of 0.5 to 15 mg. per cent the authors obtained a reproducibility of 4.6 per cent in duplicate readings. A recovery of 95.5 per cent ± 3.6 per cent was obtained with known amounts added. G.J.H.

Boger, William P.; Strickland, S. Clyde; Wright, Lemuel D.; and Ciminera, J. L.: DIABETES MELLITUS AND SERUM VITAMIN B_{12} CONCENTRATIONS: 333 PATIENTS. Proc. Soc. Exper. Biol. & Med. 96:316-19, November 1957.

Single serum samples were obtained from fasting patients and were assayed for their B_{12} content by the Lactobacillus leichmannii method. The results of this study failed to show significant differences between 333 diabetic patients and 420 normal persons. There was no statistical difference between forty-five diabetic patients with retinopathy and 138 without retinopathy. Apparently a slight difference has been demonstrated in that female diabetic patients as a group tend to have a slightly higher serum B_{12} level than the male. No correlation between vascular degenerative disease and B_{12} serum levels was noted in this study. G.J.H.

Buschmann, G.; Fritze, E.; and Marsch, A. (Dept. of Medicine, University of Göttingen, Germany): OBSERVATIONS ON THE COURSE OF DIABETES IN 1500 PATIENTS. Deutsche med. Wchnschr. 83:1284-89, July 25, 1958.

The authors present a statistical analysis of the course of the disease in 1,547 adult diabetic patients followed during the past ten years in the Diabetes Clinic of the University of Göttingen. The average duration of observation was four years. Arteriosclerotic vascular disease, of which diabetic retinopathy was the most prominent type, occurred in 30 per cent of the patients and showed no correlation with sex or severity of the disease.

The incidence of vascular complications was 70 per cent in those patients who were diabetic for twenty years and longer. Moreover, poor diabetic control was rather common among the patients with vascular disease: 42 per cent of all poorly or irregularly controlled patients had vascular complications, but only 19 per cent of those who were well controlled. Among patients of at least twenty years' standing, 77 per cent of those poorly controlled and 40 per cent of those well controlled developed vascular disease.

Obesity was present in 53 per cent of the whole group; only 11 per cent were underweight. Of the obese diabetics, 34 per cent showed vascular changes, but only 18 per cent of the underweight group. Other diabetic complications, such as

tuberculosis, infections and liver damage, varied in their incidence with the degree of metabolic control but seemed to depend on the severity of the disease. Fatal diabetic coma occurred in less than 2 per cent. Careful diabetic control, with diet, insulin and weight control, is considered the best method of avoiding the severe vascular complications which presently is the most frequent cause of death of the diabetic. M.G.G.

Chen, K. K.; Anderson, Robert C.; McCowen, Max C.; and Harris, Paul N. (Lilly Research Labs., Eli Lilly and Co., Indianapolis, Ind.): PHARMACOLOGIC ACTION OF HYPOGLYCIN A AND B. J. Pharmacol. & Exper. Therap. *121*:272-85, November 1957.

This is an interesting report of the pharmacological actions of two substances isolated from a pear-sized fruit from the tree Blighia sapida which is cultivated in Jamaica and Southern Florida. Poisoning in man from this fruit has been reported after the ingestion of the unripe fruit. In Jamaica the illness has been vaguely described as "vomiting sickness" and has been demonstrated to be associated with hypoglycemia. Two peptides, hypoglycin A and B, have been isolated from the seeds of both the ripe and unripe fruits. The administration of these substances to mice, rats, or monkeys results in a definite hypoglycemia. Cats, dogs, and pigeons appear to be refractory. Associated with the hypoglycemia, the animals developed a fatty metamorphosis of the liver associated with a reduced glycogen concentration in the liver. The administration of these drugs is associated with severe vomiting that is not correctable by the administration of glucose. The authors conclude that the mechanism of the hypoglycemic action is not similar to that of the insulin. G.J.H.

Cook, P. H. (Dept. of Labor and National Serv., Prince Henry's Hosp., Melbourne, Australia): THE EMPLOYMENT OF THE DIABETIC. M. J. Australia *1*:320-23, March 8, 1958.

Experience indicates that the principal cause for dissatisfaction in relation to employment in Australia is not that most diabetics are unable to obtain some form of employment, but rather that their career prospects are seriously prejudiced because sometimes they are unable to obtain permanent appointment in some of the major employing organizations in Australia. Permanency is tied to acceptance for superannuation or provident fund schemes, from which diabetics may be excluded simply because they are labeled diabetic. This may well mean in a public service organization, for example, that a diabetic, if he is appointed at all, may be employed only in a temporary capacity. As a result he has no assurance of continued employment, something which in his case is perhaps particularly important. If there are to be retrenchments in the service dictated by political or economic reasons, then the "temporary" is the first to go. The "temporary" may be effectively barred from promotion and the status and salary associated with such promotion, irrespective of his abilities and qualifications, because priority or preference is given to the permanent officer, who may in fact not be so well qualified for promotion. There is still much to be done if diabetics are to be permitted to take their rightful place in employment and in the community, not on the basis of a sentimental sympathy, but as a right surely based on the great weight of medical evidence. W.R.K.

DIABETES. Lancet *2*:313-15, Aug. 9, 1958.

An account is given of the third congress of the International Diabetes Federation, which was held in Düsseldorf from July 21-25, 1958. Discussion topics of great interest included: The Action of Insulin, Insulin Assay, Diabetic Angiopathy, Intermediary Metabolism in Diabetes, Diabetes and Pregnancy, Oral Hypoglycemic Drugs, A New Insulin (preparation which may be an improvement on the Lente series), and Detection of Diabetes. C.A.R.

Diamant, E. J. (Lab. of Nutrition, Dept. of Biochemistry, The Hebrew University - Hadassah Med. Sch., Jerusalem, Israel): CARBOHYDRATE METABOLISM IN EMETINE-POISONED RATS. J. Pharmacol. & Exper. Therap. *122*:465-73, April 1958.

The livers of both fed and fasted emetine-treated rats contain less glycogen than those of pair-fed control. In vitro glycogen synthesis from glucose is markedly depressed in liver slices from fasted emetine-treated animals and completely inhibited when pyruvate is used as a substrate. Gluconeogenesis in the presence of pyruvate is also lowered after such treatment. There is significant decrease in the phosphorylase and aldolase activities and homogenates prepared from livers of emetine-treated rats. The author's findings seem to indicate that the synthesis of glycogen as a main metabolic fuel for normal cell function is significantly depressed by emetine. G.J.H.

Ditzel, Jørn; and White, Priscilla (185 Pilgrim Rd.; Joslin Clin.; and Baker Clin. Res. Lab., New England Deaconess Hosp., Boston, Mass.): CAPILLARY FRAGILITY AND CONJUNCTIVAL VASCULAR CHANGES IN RELATION TO RETINOPATHY IN YOUNG DIABETICS. Angiology *9*:60-62, April 1958.

This study compares the capillary fragility index and the vascular changes visualized in the bulbar conjunctivæ of young diabetic patients. Eighty-two simultaneous estimates of capillary fragility index and observation of conjunctival vessels were made in thirty-eight pregnant patients. All had normal blood pressure, eight diabetic retinopathy, and nine persistent proteinuria. Capillary fragility was measured by the method of Barnes. Biomicroscopy and photography of the conjunctival vessels were done by the method of Ditzel and St. Clair. No vascular changes in the bulbar conjunctivæ could be correlated with the presence of an increased capillary fragility index. R.W.S.

Editorial. DETECTION OF INSULIN. Brit. M. J. *1*:938, April 19, 1958.

In a recent murder trial the case for the prosecution was based on expert evidence that a substance with the properties of insulin was present in the tissues of the dead woman. Since there are no direct chemical methods for the detection of insulin, it is at present only possible to say that a particular substance has insulin-like activity. Assay technics depend on the ability of insulin to cause hypoglycemia in test animals. While these are suitable for pharmacological purposes, they are not sufficiently sensitive to detect the minute quantities of the hormone that occur in the body under physiological conditions. It is surprising how little is known about the fate of insulin in the body. Experiments based on the injection of insulin are difficult to interpret, because, once hypoglycemia develops, efficient compensatory mechanisms come into play. What happens to insulin and how much is present at any one time are not merely questions of academic interest. It is becoming increasingly clear that only a small proportion of patients with diabetes do not secrete insulin.

In most cases some insulin is present, and this finding may indicate enhanced destruction by enzyme systems, or antago-

nism by the diabetogenic hormones, or inactivation by mechanisms similar to the insulin-binding antibody recently described. Unfortunately attempts to follow the fate of large doses of injected insulin are chemically difficult to carry out, and in themselves upset the body's normal functioning. Present methods of labeling the hormone are technically unsatisfactory because of instability of the radioactive compound. The chemical synthesis of insulin, together with the incorporation of an isotype such as sulphur^{35}, may one day provide a method by which its fate can be studied more accurately. W.R.K.

Editorial. HYPOPHYSECTOMY AND DIABETES. Brit. M. J. *2*: 296, Aug. 2, 1958.

The author discusses the vascular complications of diabetes, especially those affecting the eyes and kidneys, as the outstanding remaining problem in an otherwise controllable disease. He states several reasons why bilateral adrenalectomy or hypophysectomy might be expected to benefit the patient with severe diabetes. Reference is made to a recent publication (Brit. M. J. 2:752, 1955) on the results of hypophysectomy in twenty patients with severe diabetic complications. Although the operation was technically difficult because of cerebral vascular changes and the mortality high, the degenerative processes did seem to be arrested in some of the patients who survived. Other reports of such cases are also cited. Mention is made of the fact that not all patients have benefited from this operation and that it would be unwise to infer that this is the treatment of choice for the young diabetic patient with progressive complications. Alternative and safer ways of suppressing pituitary-adrenal function will undoubtedly be found, but the main goal must surely be to establish the reason an increasing number of diabetics suffer from serious vascular lesions. C.A.R.

Elrick, H.; Arai, Y.; and Hlad, C. J., Jr. (Radioisotope Serv., Veterans Admin. Hosp., and Dept. of Med., University of Colorado Sch. of Medicine, Denver, Colo.): THE ACTION OF GLUCAGON-INSULIN MIXTURES IN DIABETIC PATIENTS. J. Clin. Endocrinol. *18*:825-33, August 1958.

The authors present data that demonstrate an increased glucose uptake in the fore-arm with a combination of glucagon and insulin as compared to insulin alone. Analysis of the response of arterial glucose levels to the glucagon-insulin mixtures reveals that fasting blood glucose levels and the ratio of glucagon to insulin in the mixture that is injected are important factors in this response. Stabilization of the arterial glucose levels occurred consistently when the fasting level was greater than 170 mg. per 100 ml. The ratio of glucagon to insulin in the mixture was between the ranges of 0.26 to 0.90. Potential usefulness of this combined therapy is suggested but not actually demonstrated by the authors. G.J.H.

Elrick, H.; Huffman, E. R.; Hlad, C. J., Jr.; Whipple, N.; and Staub, A. (Radioisotope and Med. Serv., Veterans Admin. Hosp., and Dept. of Med., University of Colorado Sch. of Medicine, Denver, Colo.): EFFECTS OF GLUCAGON ON RENAL FUNCTION IN MAN. J. Clin. Endocrinol. *18*:813-24, August 1958.

The authors have previously shown that glucagon caused a marked and sustained increase in the renal excretion of sodium, potassium, chloride, inorganic phosphorus and radioiodine in the dog. These studies were repeated in twenty normal men. Again, an increased appearance of sodium, chloride, potassium, and inorganic phosphorus was demonstrated with suggestive evidence that this is probably due to a direct tubular effect of glucagon. As controls the authors infused glucose to mimic the hyperglycemia produced by the glucagon and were unable to produce the alteration in electrolyte clearance, thus eliminating the hyperglycemic factor as a cause of this mechanism. The one major difference between the animal and human experiments was a fact that in the human there was no increased renal clearance of I^{131}. G.J.H.

Ferner, H. (Dept. of Histology, University of the Saarland, Homburg, Germany): ON PROXIMAL ACTIONS OF HORMONES. Deutsche med. Wchnschr. *83*:1468-70, 1958.

The localization of endocrine cells within certain exocrine glands, e.g., in the testes and the pancreas, suggests a functional relationship between hormonal and exocrine systems of these glands. The author finds some evidence of such relationship through his studies of the capillaries in testes and pancreas. In both glands the same capillaries bathe first the endocrine cells and then have intimate contact with the neighboring exocrine cells. In the testes one can differentiate between an endocrine part of the capillaries and a subsequent part which supplies the testicular tubules. In the insulo-acinar capillary system of the pancreas, one finds an afferent arteriole supplying the islets of Langerhans, but instead of an efferent venule, there are numerous efferent capillaries which extend from capillary system of the islets into the peri-insular acini. This arrangement provides that a very high concentration of hormone reaches the neighboring cells. The hormone thus can exert a specific effect on these cells. In the testes high concentrations of androgens immediately reach the tubular epithelium and may influence spermatogenesis. In the pancreas the high insulin concentration of the efferent capillaries appears to be responsible for the increase of zymogen granules in the peri-insular acini. An increase of the zymogen granules in acinar cells proximal to the islets has been demonstrated if insulin secretion is stimulated by biguanidine or arylsulfonylureas. On the other hand, in endogenous insulin deficiency, as in diabetes mellitus, a pancreatic hypoplasia may be observed. The author raises the question whether an interdependence exists between the production and activation of pancreatic enzymes and the concentration of insulin in the efferent capillaries of the islets. M.G.G.

Forist, Arlington A.; Miller, William L., Jr.; Krake, John; and Struck, William A. (Res. Labs., Upjohn Co., Kalamazoo, Mich.): DETERMINATION OF PLASMA LEVELS OF TOLBUTAMIDE (1-BUTYL-3-*p*-TOLYLSULFONYLUREA, ORINASE). Proc. Soc. Exper. Biol. & Med. *96*:180-83, October 1957.

The technic for the determination of tolbutamide in plasma is given. The method consists of a chloroform extraction of weakly acidified plasma, concentration of the extract to dryness, the solution of the residue in a measurement of the absorbence of the filtrate at 228 mμ in an ultraviolet spectrophotometer. The range of effectiveness of this technic ranges from 1 to 25 mg. per 100 ml. which is well beyond the usual therapeutic range used in humans. G.J.H.

Frazer, S. C. (Edinburgh, Scotland): MISLEADING URINE TESTS. Brit. M. J. 2:566, Aug. 30, 1958.

The correspondent points out a few sources of false-positive urine tests with Tes-Tape. He mentions the possibility of contamination of the urine container by peroxide, hypochlorites, or glucose. Hypochlorites may be a constituent of household cleansers used to wash such containers. C.A.R.

Free, Alfred H.; and Fancher, Otis E. (Miles-Ames Res. Lab., Elkhart, Ind.): URINE PROTEIN TESTS IN PRESENCE OF TOLBUTAMIDE METABOLITE. Am. J. M. Tech. *24*:64-65, January-February 1958.

Tolbutamide metabolite which causes false positive reactions with common turbidity tests for protein does not react with the new colorimetric tablet test or dip-test for proteinuria. These colorimetric tests do readily react with protein in the presence of tolbutamide metabolite. W.R.K.

Fulton, Richard L.; and Bell, George E. (Dept. of Medicine, Ohio State University, Columbus, Ohio): MANAGEMENT OF DIABETES WITH TOLBUTAMIDE (ORINASE). Ohio M. J. *53*: 1288-91, November 1957.

The authors present results of clinical control in twelve patients on tolbutamide and considered it to be effective and worth while. G.J.H.

Gerstenberg, E.; Hasselblatt, A.; and Schmidt, G. (Dept. of Pharmacology, University of Göttingen, Germany): THE EFFECT OF LARGE DOSES OF TOLBUTAMIDE ON NEUROMOTOR FUNCTIONS, PARTICULARLY OF THE SPINAL CORD. Arch. exper. Path. u. Pharmakol. *231*:407-18, 1957.

The authors investigated the mechanism of the neurotoxic effects of excessively large doses of tolbutamide. The hypoglycemic sulfonylureas are known to cause irreversible muscular paralysis if given in large doses. This paralysis may occur without any hypoglycemic effect and may be accompanied by hyperglycemia. Studies were carried out on the polysynaptic reflexes of the spinal cord and on the indirectly stimulated muscles of decerebrated, decapitized and despinalized cats. The animals were anesthetized with ether and kept under artificial respiration. The sulfonylurea preparation was infused at constant rate through the jugular vein. It was found that doses of 300-500 mg./kg. paralyzed the polysynaptic reflexes and that this paralysis is due to a direct action on the spinal cord. Still higher doses (about 720 mg./kg.) decrease the contractions of the indirectly stimulated muscle while the direct stimulation is not affected.

This depression or paralysis of the reflexes was independent of the blood sugar level. Extremely large doses are fatal through paralysis of the respiratory and/or the circulatory centers of the medulla. M.G.G.

Hasselblatt, A.; and Schuster, R. (Dept. of Pharmakol., Univ. of Göttingen, Germany): EFFECT OF MEGAPHEN (CHLORPROMAZINE) AND VERONAL ON THE HYPOGLYCEMIA INDUCED BY RASTINON. Klin. Wchnschr. *36*:814-19, Sept. 1, 1958.

Phenothiazin derivatives as well as barbiturates are known to cause hyperglycemia. It appeared therefore of interest to investigate the influence of these substances on the action of hypoglycemic agents. Chlorpromazine and Veronal were selected for this study. Both agents were infused intravenously at a constant rate into fasting nonanesthetized rabbits which were given simultaneously separate infusions of insulin or of tolbutamide. The doses were: 72 mg./kg./hr. tolbutamide, 0.3 U/kg./hr. regular insulin, 10 mg./kg./hr. chlorpromazine and 100 mg./kg./hr. Veronal. Control animals received infusions of insulin or of tolbutamide. Insulin-hypoglycemia was not affected by either chlorpromazine or Veronal. Tolbutamide-hypoglycemia, on the other hand, was significantly inhibited by simultaneous administration of either of the substances. In experiments with glucose loading (500 mg./kg./hr. for two hour-periods) simultaneous infusion of chlorpromazine caused a higher peak of the blood sugar level and a markedly delayed return to the initial value, while the similar effect of Veronal was less marked. On the basis of these results and of relevant data from the literature, the authors assume that chlorpromazine and Veronal diminish the reactivity of the insulin producing islet cells, but hold it also possible that hepatic glycogenesis is inhibited. M.G.G.

Heale, T. A. F. (St. Vincent's Hosp., Melbourne, Australia): THE PLASMA KETONE TEST—ITS VALUE IN DIAGNOSIS AND TREATMENT. M. J. Australia *1*:325-27, March 8, 1958.

A knowledge of the level of ketonemia is invaluable in the treatment of diabetic acidosis, for the insulin requirements are much more closely related to it than to the blood sugar content. The more severe the ketonemia, the larger will be the total amount of insulin used and also the amount of insulin needed in the early hours of treatment.

Repeating the dilution tests for ketonemia at intervals of two hours is a reliable guide to the response to treatment and the need for further insulin. W.R.K.

John, Henry J. (University, St. Luke's, and Huron Road Hosps., Cleveland, Ohio): INFORMATION AND MISINFORMATION GAINED FROM FASTING BLOOD SUGAR ALONE IN DIABETES THERAPY. Ohio M. J. *53*:1284-87, November 1957.

The author presents detailed graphic charts on three patients which illustrate the discrepancies obtained by the use of the fasting blood sugar alone. This may lead to a gross misconception by the physician of the patients' real status. The author uses blood sugars three times daily before meals in re-evaluating the control of a diabetic patient. G.J.H.

Koenig, Robert P. (University of Cincinnati College of Medicine, Cincinnati, Ohio): MANAGEMENT OF PREGNANT DIABETICS (A TEN-YEAR SURVEY OF THE RESULTS OF DIABETES COMPLICATED BY PREGNANCY IN TWO PRIVATE GENERAL HOSPITALS—53 CASES). Ohio M. J. *53*:1297-1301, November 1957.

The results of the follow-up studies in fifty-three cases of pregnant diabetic women are presented. The patients were accumulated from two distinct geographic areas of the United States, and the method of treatment and the resulting complications in the pregnant diabetic are compared. In one group of nineteen were one maternal mortality and one infant mortality. The balance had an infant mortality of 20 per cent. The author concludes that teams of obstetricians and internists are essential to the adequate control of diabetes complicated by pregnancy. Detailed statistics and data are presented to support his conclusions. G.J.H.

Little, Brian; Vance, Vernon K.; and Rossi, Evangeline (Depts. of Obstetrics of Harvard Med. Sch. & Boston University Sch. of Medicine, & Prenatal Metabolic Project at the Boston Lying-in Hosp. and Boston City Hosp., Boston, Mass.): PLASMA 17-HYDROXYCORTICOSTEROID LEVELS IN PATIENTS WITH ABNORMAL GLUCOSE TOLERANCE DURING PREGNANCY. J. Clin. Endocrinol. *18*:49-53, January 1958.

The study has been made of the plasma 17-hydroxycorticosteroid levels in 120 women during pregnancy, at delivery, and post partum. In eighty-three of these patients there was an abnormal glucose tolerance during pregnancy, but no clinical manifestations of diabetes mellitus occurred in this group. Forty-one of these patients, with abnormal glucose tolerance, were treated with 15 units of NPH insulin daily in spite of

the absence of any clinical manifestations of diabetes. No difference in plasma levels of 17-hydroxycorticosteroid was found between the controls—the patients with abnormal glucose tolerance who were untreated, and those with abnormal glucose tolerance who were treated with insulin at any of the various stages in which assays were made before and after delivery. The authors conclude that there is no relationship between plasma levels of 17-hydroxycorticosteroid and the presence of abnormal glucose tolerance tests during pregnancy, and that insulin has no effect upon plasma 17-hydroxycorticosteroid concentration in the maternal blood. G.J.H.

Maddox, J. Kempson (Sydney, Australia): DIABETES IN EMPLOYMENT: MEDICAL ASPECTS. M. J. Australia *1*:323-24, March 8, 1958.

There may be some individual employers who would reject employment of an individual wholly on the grounds of his diabetes, but there are certain services and professions which slam the doors in their faces. Many diabetics are excellent teachers of others, and the teaching profession, which always seems to be short-handed, should not exclude them. The fighting services, police, fire services, merchant marine, etc., are of course correct in such an attitude; but the nursing profession has no more justification for refusing them entry than the medical or legal profession, or even the church. There may be some justification for modifying superannuation benefits; but civil services banning diabetics at the present time should take a lesson from the insurance world and make individual adjustments according to the age, severity of diabetes and degree of cooperation of the person concerned. There are very few jobs a diabetic can not do, and that he will often do better than others. W.R.K.

Marks, Leon J.; Weiss, Daniel M.; Leftin, Jehaudah H.; and Rossmeisl, Elsie C. (Psychiatry & Neurol. Serv. & Steroid Res. Lab., Veterans Admin. Hosp., Boston, Mass.): THE ADRENOCORTICAL RESPONSE TO INSULIN COMA. I. EFFECTS OF AN ENTIRE COURSE OF INSULIN COMA THERAPY ON THE URINARY EXCRETION OF 17-HYDROXYCORTICOSTEROIDS AND 17-KETOSTEROIDS AND ON CIRCULATING EOSINOPHILS. J. Clin. Endocrinol. *18*:235-45, March 1958.

Adrenocortical function was measured before, during, and after complete course of insulin coma therapy in a paranoid schizophrenic patient. During the days on which insulin coma therapy was administered, a moderate increase in urinary corticosteroid excretion was consistently observed which was not evident on the days that insulin coma was not produced. The 17-ketosteroid urinary excretion did not parallel the corticosteroid response. Insulin coma therapy apparently did not alter adrenocortical responsiveness to intravenous ACTH. Following the completion of the course of insulin coma, both urinary corticosteroid and 17-ketosteroid excretions remained at lower levels than during the pre-insulin control period suggesting that adrenocortical function may be depressed by a course of insulin therapy. G.J.H.

Meyers, V. W.; McCarthy, H. H.; and Wilhelm, C. M. (Depts. of Physiol., Pharmacol., and Surg., Creighton University Sch. of Medicine, Omaha, Neb.): ACTIVITY OF THYROID GLAND DURING FASTING AND REALIMENTATION WITH DIETS HIGH IN CARBOHYDRATE. Proc. Soc. Exper. Biol. & Med. 97:731-33, April 1958.

The observation that after prolonged fasting and realimentation with diets extremely high in carbohydrate in dogs, there was a high systolic pressure, rapid pulse, with a low or normal diastolic pressure, suggested the possibility that this effect might be mediated through the thyroid gland. Activity of the thyroid glands of dogs determined by serial PBI and I^{131} uptakes, was not significantly altered either during the period of prolonged fasting or the period of realimentation with diets high in carbohydrate. Therefore this cardiovascular effect is not apparently mediated by the thyroid gland. G.J.H.

Moriwaki, T.; Shigeta, Y.; Wada, M.; Oji, K.; and Yoshida, T. (First Dept. of Internal Medicine, Osaka University Med. Sch., Osaka, Japan): QUANTITATIVE STUDY OF URINARY EXCRETION OF KETONE BODIES AND ITS SIGNIFICANCE IN THE MANAGEMENT OF DIABETES MELLITUS. M. J. Osaka Univ. *8*:455-64, February 1958.

In Japan, qualitative test for ketone bodies is usually negative in diabetic patients, although late complications have been found rather often in patients with diabetes mellitus of long duration. Therefore urinary excretion of ketone bodies was studied quantitatively to evaluate the degree of metabolic control more precisely. Before any type of treatment, patients show an increased excretion of urinary ketone bodies quantitatively even though the qualitative test is negative.

After insulin treatment, excretion of urinary ketone bodies decreased sharply, but often did not become completely normal in patients whose urinary excretion of sugar ceased. Mesoxalic acid caused slight decrease in ketone body excretion. Cocarboxylase and pantothenic acid also caused slight decrease in ketone body excretion, although nicotinamide caused increase in urinary excretion of ketone bodies. Vitamin B_1 showed no influence upon ketone body excretion.

Sulfonylureas may not only be ineffective in diabetic ketosis but also may frequently be ineffective in latent ketosis. The possible value of quantitative estimation of urinary ketone bodies in deciding the optimal amount of dietary carbohydrate was also discussed. W.R.K.

Muirhead, E. E.; and Jones, F. (Dept. of Pathology, the University of Texas Southwestern Med. Sch., Dallas, Tex.): LOWERED GLUCOSE UTILIZATION, PHOSPHATE UPTAKE, AND REDUCED GLUTATHIONE CONTENT OF ERYTHROCYTES FOLLOWING BILATERAL NEPHRECTOMY. J. Lab. & Clin. Med. *51*:49-52, January 1958.

The authors present data that demonstrate a decreased glucose utilization, phosphate uptake, and glutathione content of erythrocytes following bilateral nephrectomy in the dog. These data are presented to explain the hemolytic state and shortened life span of erythrocytes in this state. G.J.H.

Murray, David B.; and Ditzel, Jørn (Baker Clin. Res. Lab., New England Deaconess Hosp., Joslin Clin. and Dept. of Med., Harvard Med. Sch., Boston, Mass.): THE EFFECT OF CO_2 INHALATION ON THE CONJUNCTIVAL VESSELS, BLOOD *p*H AND pCO_2 IN YOUNG DIABETIC SUBJECTS. J. Lab. & Clin. Med. *51*:370-80, March 1958.

The authors extend their previous work on venular dilatation seen microscopically in the conjunctival vessels and present the variations produced by exposing the subjects to the inhalation of 5 per cent carbon dioxide for a period of thirty minutes. In eight patients, blood *p*H, pCO_2, and plasma bicarbonate content was obtained before and after the exposure to carbon dioxide as well as the obtaining of a photograph of the conjunctival vessels. The inhalation of 5 per cent carbon dioxide resulted in venular dilatation, slowing of the erythrocyte veloc-

ity, occasional perivenular edema, and arteriolar constriction. The average decrease in arteriolar blood *p*H was 0.089 *p*H units. The average plasma bicarbonate content increased by 1.63 mEq./L. Average arteriole pCO_2 increased by 11 mm. of mercury. G.J.H.

Nugent, Mary A.; and Fleming, David G. (Dept. of Physiol., University of Kansas, Lawrence, Kan.): A MICRO METHOD FOR BLOOD SUGAR USING ANTHRONE. Am. J. M. Tech. *24*: 8-10, January-February 1958.

A micro technic for blood sugar estimation using anthrone has been described. The method is a composite of several others, and scaled to an initial blood sample of 0.05 ml. It has been used in this laboratory for over 4,000 blood sugar determinations with a reproducibility of ± 5 per cent. Its advantages lie in its speed and simplicity. After deproteinization, only one reagent is required; a minimum of equipment is needed; and no heating is necessary. W.R.K.

Phillips, Alec (London, E.9, England): MISLEADING URINE TESTS. Brit. M. J. 2:448, Aug. 16, 1958.

The author reports the occurrence of misleading false-positive urine tests with Tes-Tape and Clinistix. Two such urine specimens were in bottles previously containing hydrogen peroxide, and, even though they had been rinsed prior to use, there was sufficient soaked in the screwcap to bring about this misleading result. The author extends a note of caution concerning blind faith in the results of such tests. C.A.R.

Raphael, Stanley S.; and Lynch, Matthew J. G. (Dept. of Pathology, Gen. Hosp., Sudbury, Ont., Canada): KIMMELSTIEL-WILSON GLOMERULONEPHROPATHY: ITS OCCURRENCE IN DISEASES OTHER THAN DIABETES MELLITUS. A. M. A. Arch. Path. 65:420-31, April 1958.

Evidence has been adduced to show that the Kimmelstiel-Wilson lesions are not absolutely specific for diabetes mellitus. Occasional lesions of this type were found in the kidneys of twelve of twenty cases of necrotizing pancreatitis (diffuse or focal). Of thirty-three cases of alcoholic fatty infiltration of the liver, six showed occasional Kimmelstiel-Wilson lesions. In twenty-three instances of alcoholic portal cirrhosis, four showed early or slight renal lesions. In forty-six cases of benign hypertension, four cases showed lesions resembling the Kimmelstiel-Wilson type, while all of five cases of malignant hypertension had such glomerular changes. One of six cases of chronic glomerulonephritis showed small, but otherwise fairly typical lesions. All the cases of "nondiabetic Kimmelstiel-Wilson type glomerulonephropathy" found were of the exudative type, which may indicate the immaturity and juxta-agonal occurrence of such lesions. The study in effect emphasizes the specificity of the nodular lesion in diabetes mellitus. E.A.W.

Robertson, S. E. J. (Sydney, Australia): THE MANAGEMENT OF DIABETES MELLITUS IN CHILDREN. M. J. Australia *1*:324-25, March 8, 1958.

We have thought that one can supervise the child too carefully, and that it would be better to encourage independence of child and parent as much as possible. Too strict a routine, with insistence on rigid meal times and diet allowances, injections given to the minute, too frequent urine testing and too careful a watch on the weight chart can inculcate a spirit of fear in the parents and rebellion in the child. Routines should be suggested as a basis which can be altered according to circumstances, and not ordered as unalterable commands. W.R.K.

Rosenthal, F. D.; and Lees, F. (Royal Infirmary, Sheffield. Present Addresses: Dept. Neurol., Queen Elizabeth Hosp., Birmingham 15; Dept. Neurol., Royal Infirmary, Manchester 13, England): THYROTOXICOSIS WITH GLYCOSURIA AND ADRENOCORTICAL HYPERACTIVITY. Lancet *2*:340-42, Aug. 16, 1958.

The authors report three male patients with thyrotoxicosis in whom a high urinary excretion of 17-ketogenic steroids indicated adrenocortical hyperactivity. All three patients had glycosuria, and two had gynecomastia. On carbimazole treatment, the patients become euthyroid or hypothyroid; adrenocortical hyperactivity ceased, and the gynecomastia disappeared. There were no signs of acromegaly or of Cushing's syndrome, and the urinary excretion of gonadotrophins was normal in the two cases where they were estimated. The primary cause of the increased adrenocortical activity was apparently the thyrotoxicosis, and the adrenocortical hyperactivity was not primarily pituitary or adrenal in origin. C.A.R.

Roth, Arthur A. (Cleveland City Hosp. and Western Reserve University School of Med., Cleveland, Ohio): RENAL COLIC DUE TO NECROTIZING PAPILLITIS. Ohio M. J. *54*:492, April 1958.

Brief report of a case of necrotizing renal papillitis in which no evidence of diabetes is found. This indicates that not all cases of necrotizing papillitis are due to diabetes mellitus; and also points out that renal colic is occasionally due to this syndrome. G.J.H.

Saifer, Abraham; and Gerstenfeld, B. S. (Biochemistry Dept., Isaac Albert Res. Inst. of the Jewish Chronic Disease Hosp., Brooklyn, N. Y.): THE PHOTOMETRIC MICRODETERMINATION OF BLOOD GLUCOSE WITH GLUCOSE OXIDASE. J. Lab. & Clin. Med. *51*:448-60, March 1958.

The authors have adapted the enzyme glucose oxidase in a method for the microdetermination of blood glucose. They were unsuccessful in applying the technic directly to plasma or serum because of the apparent presence of inhibitory substances such as uric acid and ascorbic acid. Comparative figures obtained by using the Nelson-Somogyi technic and the glucose oxidase technic on the same fasting plasma specimens were obtained. The major advantage of the oxidase technic is that true glucose levels are obtained as opposed to the inclusion of some nonglucose reducing substances as determined by the Nelson-Somogyi method. In normals the oxidase method gives values 9 mg. per cent lower; in diabetics 20 mg. per cent lower than the Nelson-Somogyi method. G.J.H.

Seifert, G. (Dept. of Pathology, University of Leipzig, Germany): TYPES OF DIABETES MELLITUS AND THE ISLET CELL SYSTEM. Deutsche med. Wchnschr. *83*:1289-94, July 25, 1958.

The author describes a classification of diabetes which correlates the clinical features with the morphological picture of the islets of Langerhans. He differentiates three forms. These are: (1) the insulin deficiency diabetes ("type maigre") with severe metabolic disturbances, as marked hyperglycemia, tendency to acidosis, ketosis and coma, and rapid loss of weight. Here the morphological islet cell changes are significant: marked decrease in the alpha/beta cell ratio, absolute decrease in beta cell mass, degranulation of the cytoplasm and changes in the shape of the nuclei of the beta cells. (2) The senile diabetes ("type gras") with obesity, hypertension and tendency to vascular disease, with low insulin requirement and good response to oral hypoglycemic drugs. Here the morphological changes of the beta cells are minimal, but there is severe islet

cell hyalinization resulting from the deposition of acid mucopolysaccharides, and a hyperplasia of the ducts; the excretory pancreatic function and the liver and bile ducts are frequently affected too, and there is evidence of latent or manifest adrenal hypercorticism. (3) Steroid diabetes in which metabolic disturbances of the anterior pituitary and adrenal cortex predominate. Here changes in the islets are at first minimal, but after some time, the islets show evidence of overstimulation and of insufficiency. The morphological islet cell changes in steroid diabetes are evidently secondary but so appear also the changes in the closely related senile diabetes. M.G.G.

Shigeta, Y.; Unno, H.; Wada, M.; Oji, K.; and Yoshida, T. (First Dept. of Internal Medicine, Osaka University, Med. Sch., Osaka, Japan): THE METABOLISM OF α-KETO ACID AND THE ACETYLATION OF *p*-AMINOBENZOIC ACID IN DIABETES MELLITUS. M. J. Osaka Univ. *8*:465-71, February 1958.

In Japan, late vascular complications of diabetes mellitus are found rather frequently in elderly diabetics and diabetics of long duration, although there are few patients showing positive qualitative test for urinary ketone bodies except those with acute complications such as infection. It is often difficult to control these patients strictly, especially in outpatient clinics, because the amount of urinary sugar is frequently small. Therefore laboratory data other than blood glucose level and the amount of urinary sugar seemed to be of value in the metabolic control of these patients. In the present study, the increase of urinary α-keto acid following the intravenous administration of fructose and the acetylation of *p*-aminobenzoic acid (PAPA) before and after various types of treatment were studied in diabetes mellitus. The increase of urinary pyruvate and α-ketoglutarate following intravenous administration of fructose and the acetylating activity of *p*-aminobenzoic acid were studied. The increase in urinary pyruvate and α-ketoglutarate was greater in uncontrolled diabetics compared with normal adults. The acetylating activity was also depressed in diabetics. Insulin treatment improved these abnormalities remarkably. Cocarboxylase and pantothenic acid were also effective, although hypoglycemic sulfonylureas often failed to improve these abnormalities. The value of these tests in the metabolic control of diabetes mellitus was discussed. W.R.K.

Shohl, Jane; and Field, James B. (Nat'l. Institute of Arthritis & Metabolic Diseases, U. S. Dept. of Health, Education, and Welfare, Bethesda, Md.): INSULIN BINDING IN VITRO BY LEUKOCYTES FROM NORMAL AND DIABETIC SUBJECTS. J. Lab. & Clin. Med. *51*:288-92, February 1958.

The authors measured the in vitro binding of insulin to white blood cells obtained from patients with various types of diabetes, leukemia, and in normal controls .No significant differences were obtained among any of these groups. The clinical differences in the various types of diabetes do not seem to be related to the white blood cells' ability to bind insulin. G.J.H.

Terner, Charles (Worcester Foundation for Experimental Biology, Shrewsbury, Mass.): PYRUVATE AND GLUCOSE AS PRECURSORS OF ACETOACETATE. Proc. Soc. Exper. Biol. & Med. 96:801-03, December 1957.

The author, by use of the Warburg technic and C^{14}-labeled substrates incubated with homogenates of mammary gland and of kidney cortex, has demonstrated the ketogenic potency of acetate, pyruvate, glucose and lactate by measuring the rate of incorporation of the C^{14} into carrier-acetoacetate. Acetate and pyruvate were strongly ketogenic, whereas glucose and lactate gave rise to small amounts of acetoacetate. When glucose was added to homogenates, metabolizing the acetate, no antiketogenesis was observed. The postulated mechanism for those differences in capacity to produce acetoacetate is related to the rate of regeneration of reduced pyridine nucleotide. G.J.H.

Vallance-Owen, J.; Dennes, Elizabeth; and Campbell, P. N. (Postgraduate Med. Sch. of London, W.12; Middlesex Hosp. Med. Sch., London, W.1, England): INSULIN ANTAGONISM IN PLASMA OF DIABETIC PATIENTS AND NORMAL SUBJECTS. Lancet *2*:336-38, Aug. 16, 1958.

The authors report the results of their investigation of insulin antagonism in plasma of diabetic patients and normal subjects. Insulin antagonism has been found to reside in the albumin fraction of plasma from uncontrolled insulin-requiring diabetic patients and from normal subjects. The antagonistic activity of the albumin in whole normal plasma is counteracted by some other substance—possibly insulin. At present it is not known whether the antagonism is due to albumin itself or to a substance tightly bound to it. C.A.R.

Vallee, B. L.; and Kägi, J. H. R. (Biophysics Res. Lab. of the Dept. of Med., Peter Bent Brigham Hosp. and Harvard Medical School, Boston, Mass.): ABOUT THE METABOLIC SIGNIFICANCE OF ZINC. Schweiz. med. Wchnschr. *88*:132-35, Feb. 8, 1958.

Zinc is found in most tissues of animals and plants. The average daily intake for men is 15-20 mg. of which about 450 μg. per day are excreted in urine. The zinc-content of the human adult body is estimated at 2 to 3 gm. Carbonic anhydrase, carboxypeptidase, alcohol, glutamic, and lactic dehydrogenases, all contain this metal as an integral part of their molecules. Zinc is essential for their enzymatic action. Evidently, the element participates in important biochemical and physiological processes.

The association of zinc with insulin is debatable and the physiological significance of their physico-chemical interaction remains uncertain.

In postalcoholic cirrhosis of the liver marked abnormalities of zinc metabolism, zincuria and reduced serum and liver zinc content have been observed. (German) M.G.G.

Volk, Bruno W.; and Lazarus, Sydney S. (Jewish Chronic Disease Hosp., Brooklyn, N. Y.; Dept. of Pathology of the Albert Einstein College of Medicine, Bronx, N. Y.): THE EFFECT OF VARIOUS DIABETOGENIC HORMONES ON THE STRUCTURE OF THE RABBIT PANCREAS. Am. J. Path. *34*:121-35, January-February 1958.

A study has been conducted on the effect of hydrocortisone; hydrocortisone and growth hormone; and adrenocorticotropic hormone on the histologic structure of the pancreas and on the level of the blood sugar in the rabbit. The greatest degree of hyperglycemia was obtained after the simultaneous administration of hydrocortisone and growth hormone. The lesions in the pancreas consisted of β cell degranulation and glycogenization of duct epithelium and islet β cells. The lesions became more pronounced with increasing severity and duration of the diabetic state. Degranulation usually first became apparent after two days of treatment. Glycogen in ductular epithelium usually appeared by the fifth to sixth day and glycogenization of β cells was manifest at about eight days. The earlier occurrence in ductular epithelium was in accord with previous findings. It was considered to indicate that the lesion was not a

result of functional exhaustion of β cells but rather was due to hyperglycemia and was similar to the glycogenization observed in the kidney and myocardium in diabetes. Proliferation of pancreatic ductular epithelium also occurred in all groups of animals and seemed to be related to the duration of treatment rather than the degree of diabetes. Marked ductular epithelial proliferation within islets and cystic dilatation of ductules was noted. However, there was no unequivocal evidence of the formation of new β cells from ductular epithelium. It was found, contrary to other reports, that growth hormone administered simultaneously with hydrocortisone did not prevent the development of spontaneous infection in the rabbit. E.A.W.

Wick, Arne N.; and Drury, Douglas R. (Scripps Clin. and Res. Foundation, La Jolla, and Department of Physiol., Sch. of Medicine, University of Southern California, Los Angeles, Calif.): EFFECTS OF SUPERIMPOSED NATIVE INSULIN ON DISPOSAL OF IODOINSULIN IN THE BODY. Proc. Soc. Exper. Biol. & Med. 97:514-16, March 1958.

Insulin tagged with radioactive iodine has been used extensively to study the physiology of insulin, on the assumption that this compound acts similarly to endogenous insulin. The evidence for this assumption based upon studies in eviscerated-nephrectomized rabbits is presented. Greeley found that the decay of biological activity was much slower than the decay rate of iodoinsulin in the intact animal. The present authors offer evidence to suggest that part of the tracer-labeled insulin can be sequestered by the addition of superimposed native insulin. Therefore, when studying the physiology of insulin with tracer-tagged insulin, investigators should add "native insulin" in appropriate amounts. G.J.H.

Willebrands, A. F.; Geld, H. v. d.; Groen, J.; and Bolinger, R. E. (Second Med. Serv. of the Wilhelmina-Gasthuis, Amsterdam, The Netherlands): THE ANTI-INSULIN EFFECT OF EPINEPHRINE AND ITS SIGNIFICANCE FOR THE DETERMINATION OF SERUM INSULIN BY THE RAT DIAPHRAGM METHOD. Acta Physiol. Pharmacol. Neerl. 7:147-52, 1958.

The stimulation of glucose utilization of the isolated rat diaphragm by insulin is strongly suppressed by very small amounts of epinephrine or norepinephrine; 10^{-2} gamma of epinephrine was found enough to counteract almost completely the effect of 0.5 milliunits of insulin. The significance of these findings for the determination of serum insulin by the use of the isolated rat diaphragm, especially in some cases of hyperinsulinism, is discussed. W.R.K.

Wolfson, S. K., Jr.; and Williams-Ashman, H. G. (Ben May Lab. for Cancer Res. and Dept. of Biochemistry, University of Chicago, Chicago, Ill.): ISOCITRIC AND 6-PHOSPHOGLUCONIC DEHYDROGENASES IN HUMAN BLOOD SERUM. Proc. Soc. Exper. Biol. & Med. 96:231-34, October 1957.

The authors described methods for determination of isocitric and 6-phosphogluconic dehydrogenases in human blood serum. Properties of these enzymes and factors affecting their activity in the blood sera of normal human adults are discussed. G.J.H.

Worm, M. (Stralsund, Krankenhaus am Sund, East Germany): DIABETES AND PREGNANCY; OBSERVATIONS ON 157 PREGNANCIES AND DELIVERIES. Deutsche med. Wchnschr. 83:802-06, May 2, 1958.

One hundred forty-seven diabetic mothers were observed during 157 pregnancies and deliveries from 1952 to 1957. Surgical delivery, about four to five weeks prior to term, was carried out in 75 per cent of the pregnancies and the perinatal infant mortality was 19 per cent. Since 1956 an attempt was made to avoid premature deliveries wherever possible. This was achieved by a particularly strict treatment schedule of the diabetes with establishment of normoglycemia particularly during the last trimester. Cesarean delivery was undertaken only if there were strictly obstetrical indications; induction of delivery by rupturing of the membranes was never employed prior to the twenty-eighth week. This management resulted in small but significant decrease of perinatal death to 13 per cent. The incidence of malformation among the children was 6.5 per cent. (German) M.G.G.

Yam, Tan Bock; and Wells, Ronald (Dept. of Medicine, Univ. of Malaya, Malaya): A CLINICAL TRIAL OF TOLBUTAMIDE IN 220 DIABETICS. Proc. Alumni A. Malaya 10:240-66, December 1957.

The results are described of a trial of 220 diabetics on tolbutamide. In 135 patients the control of the diabetes was good, and in a further fifty-two patients it was fair. In the remaining thirty-three cases it was poor. In 165 patients previously stabilized on insulin, the control on tolbutamide was better in ninety-five cases, the same in thirty-nine and worse in thirty-one patients. A number of toxic effects were observed, mainly in the form of gastrointestinal symptoms or skin reactions. These toxic reactions were uncommon. There appear to be no definite contraindications for tolbutamide therapy, but the authors recommend that at present insulin be used as the drug of choice in diabetes associated with severe ketosis or advanced tuberculosis. W.R.K.

Zetterström, R.; and Arnhold, R. G. (Pediatric Clinic, Karolinska Sjukhuset, Stockholm, Sweden): IMPAIRED CALCIUM AND PHOSPHATE HOMEOSTASIS IN NEWBORN INFANTS OF DIABETIC MOTHERS. Acta paediat. 47:107-12, March 1958.

Calcium and phosphate blood levels were studied in nineteen newborn infants of diabetic mothers. Of these one-half were of thirty-five to thirty-six weeks' gestation; the others were of thirty-seven to forty weeks' gestation. Seven were born spontaneously; twelve were born by cesarean section. On the basis of limited data on days one to five the authors concluded that hypocalcemia with hyperphosphatemia occurred commonly in this series. The findings were correlated with those of other authors as indications of functional immaturity, including hyperbilirubinemia, retention of fluid and sodium, and occurrence of hyaline membrane disease. They suggest that the high incidence of tremor, hyperirritability and twitching in newborn infants of diabetic mothers may be related to hypocalcemia and hyperphosphatemia. R.L.J.

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NINETEENTH ANNUAL MEETING

As previously announced, the American Diabetes Association will hold its Nineteenth Annual Meeting in Atlantic City, New Jersey, June 6-7, 1959, prior to the annual session of the American Medical Association. Hotel reservations may be secured by sending in the special card which has already been mailed to all Association members. Please complete it immediately, and mail to Chalfonte-Haddon Hall, the headquarters hotel in Atlantic City, as soon as possible.

The Annual Banquet and Social Hour will be held Saturday evening, June 6. Members, their wives (or husbands) and friends are cordially invited to attend both the Banquet and Social Hour. A reservation form for the Banquet will be mailed to members a few months prior to the meeting.

Hotel reservations for the annual meeting of the American Medical Association, which follows on June 8-12, must be made through the Atlantic City Convention Bureau by filling out the reservation form which will appear in *The Journal of the American Medical Association* in early February. If you are planning to attend the ADA Nineteenth Annual Meeting and to stay over for the AMA sessions, please state on the JAMA form (or by letter) that you would like to continue your reservation at Chalfonte-Haddon Hall for the AMA sessions.

SCIENTIFIC PROGRAM

George W. Thorn, M.D., Physician-in-Chief, Peter Bent Brigham Hospital; Hersey Professor of the Theory and Practice of Physic, Harvard University Medical School, will deliver the Banting Memorial Lecture.

Physicians and other scientists, who would like to present papers at the Scientific Sessions of the Nineteenth Annual Meeting, are invited to submit abstracts to Franklin B. Peck, Sr., M.D., Chairman of the Committee on Scientific Programs, at the national office as soon as possible. Eleven copies are requested in order to expedite review by the Committee.

A list of papers to be given at the Scientific Sessions will be published in DIABETES, and the program itself, including abstracts of the papers, will be sent out to all members before the meeting.

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1958-59 MEDICAL STUDENT-INTERN ESSAY CONTEST

All medical students, interns and physicians within two years after their graduation from medical school are eligible for the seventh Medical Student-Intern Essay Contest (1958-

59). An award of $250 will be given the author or authors of the best paper reporting original work, whether laboratory investigation or clinical observation. This prize again has been made possible by the St. Louis Diabetes Association.

The best review article or case report will win $100, double the award of previous years.

Value of the material and the method of presentation will be the criteria for judgment. Any subject relating to diabetes and basic metabolic problems may be selected.

Contestants should submit by April 1, 1959, the original and two copies of their manuscripts, typewritten and double-spaced, to: Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th St., New York 17, N. Y.

It is requested that all members of the American Diabetes Association and DIABETES subscribers encourage medical students, interns and physicians within two years after their graduation from medical school to enter this contest.

QUANTITY PRICES FOR *FACTS ABOUT DIABETES*

FACTS ABOUT DIABETES is used by many physicians, clinic and hospital staffs to supplement their instructions. The thirty-two-page booklet, published by the American Diabetes Association, offers invaluable information about diabetes both for the diabetic and the nondiabetic.

To aid those who wish to furnish booklets to classes and to new patients, the following prices have been established, offering a reduction in cost for purchase in quantity:

1-99 copies, $.25 each; 100-249 copies, $.20 each; 250-499 copies, $.195 each; 500-999 copies, $.1875 each; 1,000 copies and over, $.175 each. Order blanks are available upon request to the American Diabetes Association, Inc., 1 East 45th St., New York 17, N. Y.

VOLUME 8 BINDERS AVAILABLE

A binder for 1959 matching those for previous volumes of DIABETES is available for immediate shipment at a price of $2.25. This insert binder is sturdy and attractive and will hold six issues of the magazine, plus the index supplement. Binders for Volumes 1 through 7 also are available at the same price. Address your orders to American Diabetes Association, Inc., 1 East 45th St., New York 17, N. Y.

ADA IDENTIFICATION CARD

An identification card for diabetics, issued by the American Diabetes Association, is available. This card was developed by the Committee on Information for Diabetics, bears the official seal of the Association, and will fit the average pocket or purse wallet. The price is $.10 each in quantities of one through nine and $.05 each in quantities of ten or more, both prices including handling and shipping.

NEW MEMBERS

Active

The following were elected as of Dec. 1, 1958, and Jan. 1, 1959:

California

Cochran, Burt, Jr.	Los Angeles
Hill, Robert	Berkeley
Georgia	
Agostas, William N.	Augusta
Iowa	
Peterson, Loren G.	Des Moines
Kentucky	
Moosnick, Franklin B.	Lexington
Maryland	
Carey, T. Nelson	Baltimore
Massachusetts	
Khachadurian, A. K.	Boston
Michigan	
Breneman, James C.	Galesburg
O'Connor, Katheryn L.	Detroit
New Jersey	
Grossman, Rubin	Bayonne
New York	
Bookman, John J.	New York
Hollis, William C.	Hempstead
Morgenstern, Leo L.	White Plains
Mouratoff, George J.	New York
Tirsch, Harry	Brooklyn
Pennsylvania	
Hoch, John J.	Nazareth
Washington	
Tanner, Donald C.	Bellevue
Wisconsin	
Esser, John H.	Milwaukee

Other Countries

Cuba	
Centurion, Jose J.	Havana
De Varona, F. J.	Havana
Saladrigas, O. Montoro	Havana
Schutte, Jose Antonio	Havana
Schutte, Julio Francisco	Havana
Villaverde, Manuel M.	Havana

NEWS OF AFFILIATE ASSOCIATIONS

The BUFFALO DIABETES ASSOCIATION (Clinical Society) gave its first annual dinner meeting in Buffalo on October 29. John Sullivan, M.D., Boston, spoke on "Neurological Complications of Diabetes." A number of members of the Buffalo Neuropsychiatric Society also attended the meeting.

The FLORIDA DIABETES ASSOCIATION held its sixth annual session Thursday and Friday, October 30-31, in Miami Beach. Cooperating organizations were the Florida Medical Association, the Florida State Board of Health, and the Division of Postgraduate Education, College of Medicine, University of Florida.

Moderators on Thursday were George H. Garmany, M.D., President, Tallahassee; and George F. Schmitt, Jr., M.D., Miami. Richard H. Sinden, M.D., Miami; and Joseph J. Lowenthal, M.D., Jacksonville, were moderators on Friday.

The Thursday program included: "Insulin Resistance in Diabetic Acidosis," and "Chronic Insulin Resistance," by James B. Field, M.D., U.S. Public Health Service, National Institutes of Health, Bethesda; "Diabetic Acidosis," by Howard F. Root, M.D., Physician-in-Chief, Deaconess Hospital, and Associate Professor of Medicine, Harvard University School of

Medicine, Boston; "Some Observations Concerning the Natural History of Diabetes Mellitus," by A. Gorman Hills, M.D., Associate Professor of Medicine, University of Miami School of Medicine, Miami; "Hyperglycemic States Not Primarily Due to Lack of Insulin," by Thomas F. Frawley, M.D., Director, Department of Endocrinology and Metabolism, The Albany Medical College of Union University, Albany; and "Control of Diabetes and Its Vascular Sequelae," by Dr. Root.

These papers and speakers comprised the Friday program: "Experiences with Chlorpropamide, A New Anti-Diabetic Agent," by Dr. Lowenthal; "Mode of Action of Sulfonylureas: A Resolving Enigma," by Dr. Frawley; "The Eyes and Diabetes," by Dr. Root; "Vitamin Metabolism in Diabetes Mellitus," by Dr. Field; "Gynecomastia," by William C. Thomas, Jr., M.D., Assistant Professor of Medicine, University of Florida College of Medicine, Gainesville; and "New Clinical and Pathophysiological Knowledge of the Adrenogenital Syndrome and Its Variants," by Dr. Frawley.

The NEW ENGLAND DIABETES ASSOCIATION (Clinical Society) held a fall clinical meeting at the Dowling Amphitheatre, Boston City Hospital, on December 4. Solomon A. Berson, M.D., Veterans Administration Hospital, Bronx, New York, spoke on "Anti-Insulin Factors." The Association held a Western Massachusetts Clinical Meeting on December 9 at the Springfield Memorial Hospital in Springfield. A "Panel on Clinical Management of Diabetes" was presented by Samuel B. Beaser, M.D., and Edward A. Frank, M.D., both of Beth Israel Hospital; and Priscilla White, M.D., New England Deaconess Hospital.

The Clinical Society of the New England Diabetes Association will convene at the New England Deaconess Hospital in Boston for its spring clinical meeting in March, 1959. The annual dinner meeting will be held in June.

NEWS NOTES

NUTRITION EDUCATION PROGRAMS

The National Vitamin Foundation in cooperation with the Commissions on Nutrition of the Pennsylvania State Medical Society and the Philadelphia County Medical Society is developing nutrition education programs for practicing physicians. This meeting was held *January 10:* "Fats, Cholesterol and Atherosclerosis—A Progress Report," by Norman Jolliffe, M.D., in Atlantic City for the New Jersey Academy of General Practice.

Programs presented last fall included: *September 9:* "Nutritional Aspects in Alcoholism," by R. S. Goodhart, M.D., at Allentown-Lehigh Valley Country Club for the Lehigh County Medical Society. *October 6:* "Importance of Salt in the Diet," by John Moyer, Jr., M.D.; and "Fat Metabolism in Relation to Cardiovascular Diseases," by David Seligson, M.D., with Michael G. Wohl, M.D., as moderator, in Philadelphia for the Philadelphia County Medical Society. *October 8:* "Metabolic Response to Trauma, Tube Feeding, Jejunostomy," by R. Ravdin, M.D.; and "Nutrition Evaluation Prior to Surgical Procedures and Parenteral Feeding," by J. F. Mueller, M.D., in Wilkes Barre for the Luzerne County Medical Society. *November 13:* "General Therapeutic Nutrition with Emphasis on Arthritic Disease," by Willard Krehl, M.D., in Media for the Delaware County Medical Society. *November 18:* "Treatment of Obesity with Particular Reference to Nutrition," by Robert Hillman, M.D., in Easton for the Northampton County Medical Society. *December 3:* "Nutritional and Metabolic Aspects of Cardiovascular Disease," by Richard Vilter, M.D., in Lancaster for the Lancaster County Medical Society.

Robert S. Goodhart, M.D., is Scientific Director of the National Vitamin Foundation, and Michael G. Wohl, M.D., Chairman of the Commissions on Nutrition of the Pennsylvania State Medical Society and the Philadelphia County Medical Society.

ADA MEMBERS TO TAKE PART IN POSTGRADUATE COURSES

Ford K. Hick, M.D., will be an Associate Director of Course No. 5 arranged by the American College of Physicians, entitled "Internal Medicine—Especially Therapeutics." It will be held at the University of Illinois College of Medicine in Chicago, Jan. 12-16, 1959.

Among the Officers of Instruction for Course No. 7, "Recent Advances in Cardiovascular Diseases," to be held Feb. 9-13, 1959, at The Mount Sinai Hospital in New York, will be David Adlersberg, M.D. He will speak on "Genetic and Hormonal Factors in Atherosclerosis" and take part in a "Panel on Atherosclerosis and Coronary Thrombi."

Garfield G. Duncan, M.D., will be Director of Course No. 8, titled "Recent Advances in Internal Medicine," which will be held Feb. 23-27, 1959, at the Pennsylvania Hospital in Philadelphia.

PERSONALS

E. T. BELL, M.D., Minneapolis, delivered the third annual Carl V. Weller lecture of the Michigan Pathological Society on December 13 at Ann Arbor. In his lecture, entitled "The Clinical Course and Pathological Anatomy of Diabetes Mellitus," he reviewed his forty-six years' experience in the Department of Pathology at the University of Minnesota, where he is Emeritus Professor of Pathology. Dr. Bell pointed out that the combined use of insulin and antibiotics has resulted in a spectacular increase in the length of life of young diabetics.

The Carl V. Weller lectures were established by the Michigan Pathological Society in 1956 to honor the late Dr. Weller, Chairman of the Department of Pathology of the School of Medicine of the University of Michigan for many years. Previous addresses have been given by Dr. Howard T. Karsner, Research Adviser to the Surgeon General, U.S. Navy; and Dr. John C. Bugher, Director for Medical Education and Public Health, Rockefeller Foundation.

NECROLOGY

ROBERT M. ALEXANDER, Reading, Pennsylvania, born June 14, 1884.

EDGAR F. COSGROVE, Pittsburgh, Pennsylvania, born Aug. 30, 1909.

HARRY I. CRAMER, Montreal, Canada, born July 14, 1910.

J. C. PASS FEARRINGTON, Winston-Salem, North Carolina, born Nov. 25, 1898.

LEON JONAS, Philadelphia, Pennsylvania, born Aug. 30, 1887.

BERNARD S. OPPENHEIMER, New York City, born June 20, 1876.

IRVING SOMACH, New York City, born Feb. 10, 1901.

DAVID A. TUCKER, JR., Cincinnati, Ohio, born Dec. 21, 1890.